Pharmacological treatment with annexin A1-derived peptide protects against cisplatin-induced hearing loss.

Sena , Leticia ; Sanches , José Marcos ; Azevedo, Mariza; Sasso, Gisela ; Gil, Cristiane

2019

10th International Conference on Annexin Biology

ANNEXINS 2019

15 to 18 September 2019
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10th International Conference on the Annexin Biology

15th-18th September 2019

ANNEXINS 2019

Conference Organization:

Volker Gerke and Ursula Rescher

Institute of Medical Biochemistry - Center For Molecular Biology of Inflammation
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Dear participants,

Welcome to Münster for the 10th International Conference on Annexins (Annexins 2019).

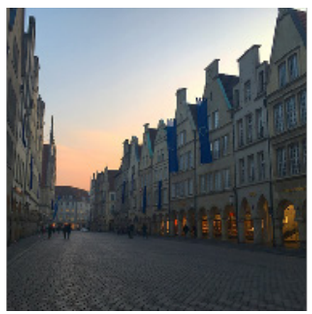
The Annexins Conference is a biannual international conference on the biology of the annexin family of Ca^{2+}-binding proteins. It has been 20 years since this conference series started, and as always, Annexins 2019 will be an outstanding international forum to present and discuss the latest advances in research, development, standards, and applications related to the annexin protein family in a relaxed setting. The vibrant program includes cutting-edge science, plenary sessions, poster sessions, and after meeting parties and banquets. We hope you have a great time!

Attending Annexins 2019 means you also will experience Münster, a beautiful city famous for the "Peace of Westphalia, a series of treaties signed 1648, ending the Thirty Years' War and the beginning of the modern international system. With over 50,000 enrolled students, Münster is one of Germany's largest university towns - a perfect blend of traditions and international flavour.

The location is perfect for a meeting: on the shore of the Aasee – an artificial lake in the heart of town. Annexins 2019 will be an exceptional conference for sharing the latest insights as well as experiencing the unique atmosphere of Münster.

We are glad to have you in Münster!

Organizing Committee, Annexins 2019


Photos: Anna L. L. Matos

Venue

1 - City Palace (Schloss), Schlossplatz 2, 48149 Münster

2 - Hier & Jetzt, Bismarckallee 11, 48151 Münster

3 - Soccer Field, Annette-Allee, 48149 Münster

4 - Schlossgarten Restaurant, Schlossgarten 4, 48149 Münster

Münster Sights

1 - St. Lamberti Church + Prinzipalmarkt

2 - Cathedral Square

3 - Liebfrauenkirche (Church)

4 - Old town hall

5 - Pub & Brewery Street

6 - Town Museum

7 - Museum of Art and cultural history

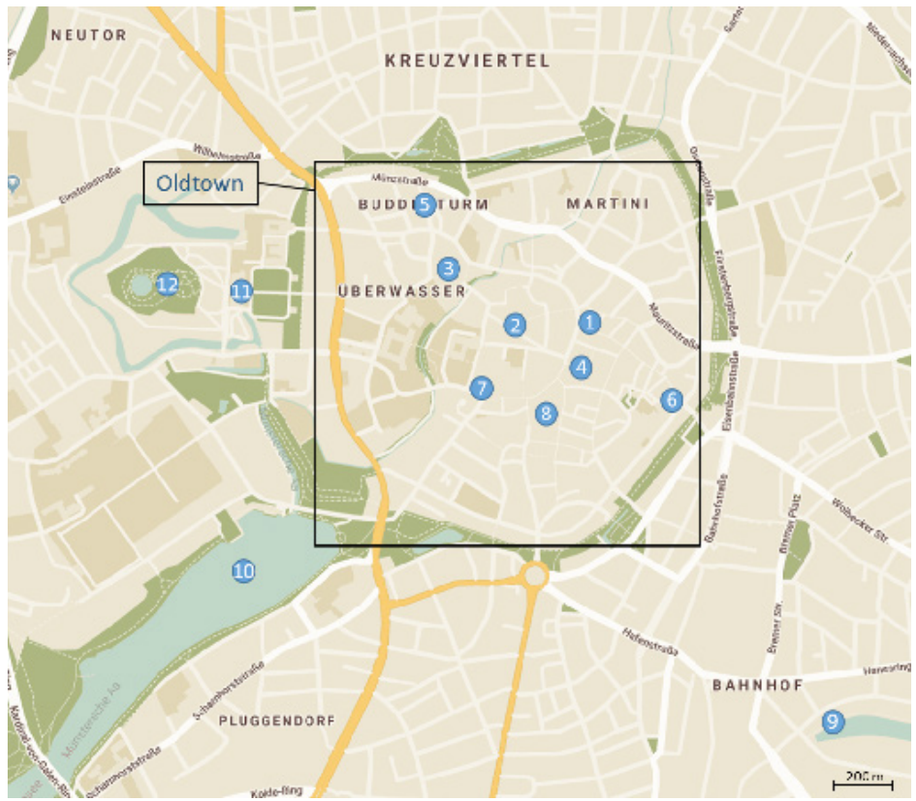
8 - Picasso-Museum

9 - Harbour

10 - Aasee

11 - Schloss + Schlossgarten

12 - Botanical garden



PROGRAM

SUNDAY, 15.09.2019

Venue: City Palace (Schloss), Schlossplatz 2, 48149 Münster.

16:00 – Registration at City Palace (Schloss)

18:00 – Welcome by Volker Gerke and Ursula Rescher

18:10 – Evening Lecture

Translating carbohydrate information into function for the clathrin-inde pendent construction of endocytic pits

Ludger Johannes, Institut Curie-Paris, France

19:30 - Opening Reception

Mix and mingle while enjoying food and drinks

MONDAY, 16.09.2019

Venue: Hier & Jetzt, Bismarckallee 11, 48151 Münster

Annexins and Cellular Organization

Session 1: Membrane dynamics and plasticity

Chair: *Carlos Enrich*

08:30 – Expression of Annexins in yeast provides protection against deleterious effects of the biofuel isobutanol
Carl Creutz, University of Virginia, USA

08:55 – Annexin A6-mediated control of endosomal cholesterol export via TBC1D15/Rab7/StARD3 axis and its multiple cellular consequences
Thomas Grewal, University of Sydney, Australia

09:20 – Annexin A8 and retinal pigment epithelial cell differentiation
Stephen Moss, University College London, UK

09:45 - Annexin A2 is a membrane lipid organizer protein
Jesus Ayala Sanmartin, ENS, Paris, France

09:55 - Annexins induce rolling and distinct morphologies in membranes with free edges
Adam Simonsen, University of Southern Denmark, Odense,

10:05 – Coffee break

Session 2: Endo- and exocytosis

Chair: *Stephen Moss*

10:30 – Phosphorylation cycling of Annexin A2 Tyr23 is critical for calcium-regulated exocytosis in neuroendocrine cells
Sylvette Chasserot-Golaz, Université de Strasbourg, France

10:55 – Annexin A6 depletion induces formation of membrane contact sites between endolysosomes and the endoplasmic reticulum in NPC1 mutant cells
Carlos Enrich, University of Barcelona, Barcelona, Spain

11:20 – Roles of AnnexinA1-dependent and annexinA1-independent membrane contacts between the ER and endocytic pathway
Clare Futter, University College London, UK

11:45 – Role of Annexin A6 in hepatic energetic metabolism in mice
Carles Rentero, University of Barcelona, Spain

11:55 - Phosphorylation of Annexin A2 is essential for its association with exosomes in triple negative breast cancer and for imparting proliferative and invasive phenotype to other cells
Priyanka Prakash Desai, University of North Texas, Denton, USA

12:05 – Lunch Break

Annexins in Health and Disease I

Session 3: Diseases (cancer, inflammation)

Chair: *Mauro Perretti*

14:00 – The Role of Annexin A1 in Resolving Thrombo-inflammation
Felicity Gavins, Brunel University London, Uxbridge, UK

14:25 – Annexin A2 Integrates Fibrinolysis with Angiogenesis and Retinal Injury
Katherine A. Hajjar, Weil Cornell Medical College, New York, USA

14:50 – The Annexin A1/FPR2 axis in pneumococcal pneumonia and polymicrobial sepsis
Lirlândia Pires de Sousa, UFMG, Belo Horizonte, Brazil

15:15 – Uterine receptivity is increased by AnxA1 via FPR and ERK
Sandra Farsky, University of Sao Paulo, Brazil

15:25 - Diverse roles of Annexin A6 in triple negative breast cancer
Amos Sakwe, Meharry Medical College, Nashville, USA

15:35 – Coffee Break and Poster Session I

19:30 - Conference BBQ at Hier und Jetzt

TUESDAY, 17.09.2019

Annexins in Health and Disease I

Session 4: Diagnosis and treatment

Chair: *Katherine A. Hajjar*

08:30 – Annexin A1 and the control of joint disease: local and distant effects
Mauro Perretti, Queen Mary University of London, UK

08:55 – Annexins as biomarkers
Chris Reutelingsperger, Maastricht University, The Netherlands

09:20 – Dectin-1 binding to Annexins on apoptotic cells induces peripheral tolerance via NADPH oxidase-2
Kevin Bode, DKFZ, Heidelberg

09:30 - The destiny of dying retinal ganglion cells in glaucoma
Wenting You, University of Maastricht, Netherlands

09:40 – Coffee break

Annexins in Health and Disease II

Session 5: Membrane repair

Chair: Annette Draeger

10:30 – Extracellular Role of Annexin A2 in muscle repair and degeneration
Jyoti Jaiswal, Children's Nationale Research Institute, Wash., USA

10:55 – Mechanisms of Annexin mediated plasma membrane repair
Jesper Nylandsted, University of Copenhagen, Denmark

11:20 – Dysferlin-mediated phosphatidylserine sorting engages macrophages in sarcolemma repair
Volker Middel, KIT, Karlsruhe, Germany

11:45 – Downregulation of Annexin A1 in human placenta may be responsible for the development of preeclampsia
Anthony Bouter, University of Bordeaux, France

11:55 - Annexin A6 in membrane repair of human skeletal muscle cells
Coralie Croissant, University of Bordeaux, France

12:05 – Lunch Break

14:00 – Poster Session II

16:00 – Soccer Game/Free Afternoon

18:30 - Reception at Schlossgarten Restaurant (Live Music)

Poster awards and group picture

19:30 – Conference Dinner at Schlossgarten Restaurant

WEDNESDAY, 18.09.2017

Annexins in Health and Disease II

Session 6: Host-pathogen interactions

Chair: *Jesper Nylandsted*

08:30 – The Annexins: evidence tape for membrane lesions.
Annette Dräger, University of Bern, Switzerland

08:55 – Heterotetrameric Annexin A2/S100A10 (A2t) is essential for oncogenic human papillomavirus trafficking and capsid disassembly, and protects virions from lysosomal degradation.
Martin Kast, University of Southern California, Los Angeles, USA

09:20 – Annexin A3: an important host factor for HCV infection
Hanna Bley, University Marburg, Germany

09:30 – Cellular survival following membrane perforation by bacterial pore forming toxins relies on microvesicle-shedding efficiency
Rene Köffel, University of Bern, Switzerland

09:40 – Annexin A8 is a major factor in dendritic cell maturation and functionality
Sebastian Schloer, University of Muenster, Germany

09:50 - Coffee break

Session 7: Annexins – Latest Developments and Future Perspectives

Chair: *Chris Reutelingsperger*

10:30 – Annexin A1 - friend or foe?
Lina Lim Hsiu Kim, National University of Singapore

10:55 – Annexin A5 in vascular diseases
Anna Frostegard, Karolinska Instituet, Solna, Sweden

11:20 – Two arabidopsis annexins function in transporting sugar to root tips to affect primary root growth
Greg Clark, University of Texas, Austin, USA

11:30 - Annexin A2 binds to the internal ribosomal entry site of c-myc mRNA in the presence of calcium to inhibit translation
Anni Vedeler, University of Bergen, Norway

11:40 - Gonadotropin releasing hormone (GnRH) regulation of ANXA5 containing bleb and microvesicle formation in the pituitary gonadotropes
Mitsumori Kawaminami, University of Science, Okoyama, Japan

11:50 - Closing Remarks and Farewell

Talk Abstracts

Translating carbohydrate information into function for the clathrin-independent construction of endocytic pits

Ludger Johannes

Institut Curie, U1143 INSERM – UMR3666 CNRS, Paris, France

Several endocytic processes do not require the activity of clathrin, and it has been a major question in membrane biology to know how the plasma membrane is bent and cargo proteins are sorted in these cases. Our previous studies have allowed us to propose a novel hypothesis, termed GlycoLipid-Lectin (GL-Lect) hypothesis: Nanodomain construction by glycolipid-binding lectins from pathogens (e.g. Shiga and cholera toxins, polyoma and noroviruses) or cells (galectins) induce narrow membrane bending and the formation of tubular endocytic pits from which clathrin-independent endocytic carriers are generated for the cellular uptake of these pathogens and pathogenic factors, or of cellular proteins (signaling receptors, adhesion molecules…). We are now identifying ways by which the GL-Lect mechanism is acutely controlled by growth factor signaling. Furthermore, we study how GL-Lect domain construction at the plasma membrane programs the intracellular distribution of cargo molecules, notably via the retrograde transport route, thereby exploiting the polarized secretion capacity of the Golgi apparatus for the distribution of cargo proteins to specialized plasma membrane domains in migratory cells (leading edge), epithelial cells (apicobasal sorting and transcytosis), and lymphocytes (immunological synapse). These studies are performed using a combination of cell biological (lattice light sheet microscopy), biochemical (membrane protein purification and reconstitution, glycosphingolipidomics), chemical biology (glycosphingolipid synthesis, small molecule screening), and structural biology (cryo-EM) techniques in model membrane systems, cells, and living organisms.

Annexins and Cellular Organization

Session 1: Membrane dynamics and plasticity

Expression of annexins in yeast provides protection against deleterious effects of the biofuel isobutanol

Carl Creutz
University of Virginia, USA

The ability of microorganisms to produce biofuels by fermentation is adversely affected by the effects of the hydrophobic biofuel on membrane structure. It is demonstrated here that heterologous expression of annexins can reduce deleterious effects of isobutanol on S. cerevisiae viability and complex membrane functions. Therefore, expression of annexins in industrial strains of yeast may prove beneficial in biofuel production. The effects of annexins A1, A5, A6, and Nex-1were determined in two types of experiments. First, yeast cultures expressing the annexins were inoculated into medium containing a concentration of isobutanol (2%) that completely blocks cell growth. After incubation for 48 hours, the viability of the yeast cells was determined by diluting the cultures into normal medium. Control cells without annexins were non-viable, while expression of the annexins allowed the cells to survive. The second experiment revealed an ability of the annexins to protect a complex membrane-remodeling event: adaptation of the yeast cell membrane that occurs during a shift from growth in glucose to galactose. This adaptation includes the insertion of galactose transport proteins into the plasma membrane. In the presence of 1% isobutanol, the annexins enhanced the initial rate of growth of yeast 3 to 4 fold after dilution of a culture grown in 2% glucose into 2% galactose.

Annexin A6-mediated control of endosomal cholesterol export via the TBC1D15/Rab7/StARD3 axis and its multiple cellular consequences

Thomas Grewal*, Jaimy Jose*, Elsa Meneses-Salas#, Carles Rentero# and Carlos Enrich#

*School of Pharmacy, Faculty of Medicine and Health, University of Sydney, Sydney, NSW 2006, Australia.
#Departament de Biomedicina, Unitat de Biologia Cel·lular, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona. and Centre de Recerca Biomèdica CELLEX, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036-Barcelona, Spain.

Annexin A6 (AnxA6) overexpression induces cholesterol accumulation in late endosomes, a phenotype reminiscent of Niemann-Pick type C1 (NPC1) mutant cells. Here, we demonstrate that this cellular cholesterol imbalance is due to AnxA6 promoting Rab7 inactivation via TBC1D15, a Rab7-GTPase activating protein (Rab7-GAP). Accordingly, AnxA6 as well as TBC1D15 depletion elevated Rab7 activity, restoring cholesterol export from late endosomes in NPC1 mutant cells. This was associated with peripheral distribution and increased mobility of late endosomes. Moreover, in AnxA6-deficient NPC1 mutant cells, Rab7-mediated rescue of late endosome-cholesterol export required the StAR-related lipid transfer domain-3 (StARD3) protein. Given the fundamental role of Rab7 in late endosomal/lysosomal functioning, this regulatory role AnxA6 for cellular cholesterol distribution and Rab7 activity has multiple consequences, affecting focal adhesion dynamics and distribution, metalloprotease secretion, but also the coordination of functions connected to the regulation of cellular metabolism, such as autophagy and mTOR signalling. Hence, alterations of AnxA6 levels in the late endosomal compartment could contribute to metabolic adaptations, such as increased demand for cholesterol, that is now well recognized as an additional hallmark of cancer.

Annexin A8 and retinal pigment epithelial cell differentiation

Stephen Moss
University College London, UK

Annexin A2 is a membrane lipid organizer protein

Jesus Ayala Sanmartin
ENS, Paris, France

Annexin A2 is a calcium and phospholipid binding protein able to associate to proteins. Several proteins such as actin or caveolin have been described as annexin A2 partners and suggested to play roles in cellular processes involving membrane and cytoskeleton dynamics. However, the best known partners of annexin A2 (and most of annexins) are the anionic phospholipids. Herein we studied different aspects of membrane lipid rearrangements induced by annexin A2 binding. By using X-ray diffraction we observed that annexin A2 has the property to stabilize lamellar structures in the absence and presence of cholesterol. By using a pyrene-labelled cholesterol probe and the environmental membrane probe di-4-ANNEPDHQ, we observed that annexin 2 is able to modify cholesterol distribution in model membranes and to induce modifications of membrane lipid order in epithelial cells. These changes in membrane order were different depending on the cellular membrane regions.
Keywords: Annexin A2; Cholesterol; Membrane domains; Membrane order.

Annexins induce rolling and distinct morphologies in membranes with free edges

Adam Cohen Simonsen
University of Southern Denmark, Odense

Eukaryotic cells have developed several strategies for recovering from disruptions to the plasma membrane. A shared feature of membrane repair, is the binding of specific repair proteins in response to damage, to remodel the plasma membrane and facilitate sealing and survival of the cell. Members of the Annexin family are well known to be involved in membrane repair as triggered by Ca2+ influx to the cytosol upon damage. But, a mechanistic insight into the precise membrane-remodeling occurring during repair is lacking. Biophysical experiments on artificial membranes designed to model features of cellular repair, can help uncovering such mechanisms.
In a planar model membrane system, we show by microscopy that curvature stress induced by annexin A4 binding leads to roll-up of the membrane as initiated from free membrane edges[2]. In a complementary approach, the rolling process is modeled theoretically, taking into account curvature stress and adhesion to the underlying surface. Measurements of human Annexins show that generation of membrane curvature is a shared feature and that distinct membrane morphologies are produced by different members of the Annexin family[2]. Our results point to membrane curvature near hole-edges as a key event in the plasma membrane repair process.
[1] Boye, T. L et. al. Sci. Rep. 2018, 8 (1), 10309. DOI:10.1038/s41598-018-28481-z
[2] Boye, T. L. et. al. Nature Com. 2017, 8 (1), 1623. DOI: 10.1038/s41467-017-01743-6

Keywords: Membrane Repair, Annexins, Microscopy, Membrane Rolling, Model Membranes

Annexins and Cellular Organization

Session 2: Endo- and exocytosis

Phosphorylation cycling of Annexin A2 Tyr23 is critical for calcium-regulated exocytosis in neuroendocrine cells

Sylvette Chasserot-Golaz
Université de Strasbourg, France

Annexin A2 (AnxA2) is involved in calcium-regulated exocytosis. After highlighting that AnxA2 bundled cortical actin to connect docked secretory granules to the plasma membrane and contribute to the formation of GM1-enriched lipid domains at the exocytotic sites in chromaffin cells, we focused on the regulation of AnxA2 by the phosphorylation of tyrosine 23 (Tyr23) that reduces the binding of AnxA2 to actin filaments and lipids. We showed that cell stimulation triggers the phosphorylation of AnxA2 on Tyr23 at exocytotic sites and that the expression of AnxA2 mutants carrying phosphorylation deficient (Y23A) or phosphomimetic mutation (Y23E) reduces the number exocytotic sites. However the expression of mutant AnxA2-Y23A inhibits the formation of lipid microdomains, while that of the AnxA2-Y23E affects the actin filaments associated with docked granule. This suggests that phosphorylation/dephosphorylation switch at Tyr23 in AnxA2 is critical for exocytosis in neuroendocrine cells. Finally, we observed the presence of the Tyr23 phosphorylated tetramer at the surface of stimulated chromaffin cells. So what is the role of extracellular AnxA2? Since AnxA2 is a co-receptor for tissue plasminogen activator (t-PA), the extracellular AnxA2 tetramer could bind the circulating plasminogen and the secreted t-PA to cleave the released chromogranin A into peptides, able to inhibit secretion. Thus, AnxA2 could participate in autocrine and/or paracrine regulation of catecholamine secretion.

Keywords: Annexin A2 - Exocytosis - Chromaffin cells - lipid domains - Actin-F – Tyr23 Phosphorylation

Annexin A6 depletion induces formation of membrane contact sites between endolysosomes and the endoplasmic reticulum in NPC1 mutant cells

Carlos Enrich
University of Barcelona, Barcelona, Spain

Annexin A6 (AnxA6), the largest member of the annexin family, has been implicated in the regulation of endo- and exocytic pathways, cholesterol homeostasis and the formation of multifactorial signaling complexes. Like other annexins, the majority of AnxA6 binds to membranes in a Ca2+-dependent manner, yet cholesterol loading of late endosomes, using the NPC1 inhibitor U18666A or low density lipoproteins (LDL), led to the recruitment of significant amounts of AnxA6 to late endosomes. In previous studies we showed that AnxA6 overexpression resulted in the accumulation of cholesterol in late endosomes. Although these studies linked AnxA6 with cholesterol export from endolysosomes, the underlying molecular mechanisms remained unclear. Now we demonstrate that AnxA6 depletion alleviates the NPC1 mutant phenotype through two critical mechanisms: it triggers endogenous Rab7 activation but it also enables StARD3 to facilitate the formation and functioning of membrane contact sites (MCS) between endolysosomes and the ER, aiding late endosomal cholesterol export. Conventional electron microscopy showed abundant lipid droplets (LD) in LDL-loaded CHO wildtype (CHO-WT) cells, but not in the CHO NPC1 mutant cell line M12. LD formation upon LDL-loading was restored in AnxA6-depleted CHO M12 cells, yet depletion of StARD3 in these cells resulted in a lack of cholesterol transfer into LD, identifying StARD3 and MCS as instrumental to transfer and store LDL-derived neutral lipids as cholesteryl esters in LD. Altogether, AnxA6 deficiency in CHO M12 cells correlated with a significant increase of surface contacts between endolysosomes and ER, to facilitate the transfer of cholesterol out of late endosomes and the relief of late endosomal cholesterol accumulation.

Keywords: Annexin A6, cholesterol, endolysosomes, membrane contact sites, NPC1

Roles of AnnexinA1-dependent and annexinA1-independent membrane contacts between the ER and endocytic pathway

Clare Futter
University College London, UK

We have identified the presence of multiple biochemically distinct membrane contacts between the ER and endocytic pathway. Annexin1:S100A11 complexes tether EGF receptor-containing multivesicular endosomes/bodies (MVBs) to the ER and are sites of interaction between the ER-localised phosphatase, PTP1B, and endosomal substrates. We are investigating the role played by these contacts in the formation of intraluminal vesicles (ILVs) within MVBs. ILV formation within EGF receptor-containing MVBs depends on the ESCRT machinery, at least two components of which are substrates of PTP1B. We are exploring the possibility that regulation of the phosphorylation state of ESCRT components plays a role in regulating ILV formation and EGF receptor traffic to the lysosome. ILV formation can also be facilitated by transport of ER-derived cholesterol to MVBs at annexinA1-dependent contacts to support ILV formation in the absence of LDL-derived cholesterol. We also have evidence supporting a role for annexinA1-independent contacts in transport of LDL-derived cholesterol in the reverse direction, from the endocytic pathway to the ER for esterification.

Role of annexin A6 in hepatic energetic metabolism in mice

Patricia Blanco-Muñoz[1], Anna Álvarez-Guaita[1], Elsa Meneses-Salas[1], Thomas Grewal[2], Carlos Enrich[1] and Carles Rentero[1].
1Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona (Spain) and [2]Faculty of Pharmacy, University of Sydney (Australia).

Liver regeneration requires the organized and sequential activation of events that lead to the restoration of hepatic mass. During this process, other vital liver functions need to be preserved, such as the maintenance of blood glucose homeostasis, balancing the degradation of hepatic glycogen stores and gluconeogenesis (GNG). Under metabolic stress, alanine is the main hepatic gluconeogenic substrate, and its availability is the rate-limiting step in this pathway. The Na^+-coupled neutral amino acid transporters (SNAT) 2 and 4 are believed to facilitate hepatic alanine uptake. In previous studies we demonstrated that Annexin A6 (AnxA6), a member of the calcium-dependent phospholipid binding annexins, regulates membrane trafficking along endo- and exocytic pathways. Yet, although AnxA6 is abundantly expressed in the liver, its function in hepatic physiology remains unknown. In this study, we investigated the potential contribution of AnxA6 in liver regeneration and glucose metabolism. Utilizing AnxA6 knock-out mice (AnxA6-/-), we challenged liver function after partial hepatectomy (PHx) inducing acute proliferative and metabolic stress. Biochemical and immunofluorescent approaches were employed to dissect AnxA6-/- mice liver proliferation and energetic metabolism. Most strikingly, AnxA6-/- mice exhibited low survival after PHx. This was associated with an irreversible and progressive drop of blood glucose levels. While the exogenous glucose administration or recombinant adeno-associated viral particle (rAAV)-AnxA6 infection rescued AnxA6-/- mice survival after PHx, the sustained hypoglycaemia in partially hepatectomized AnxA6-/- mice was the consequence of an impaired alanine-dependent GNG in AnxA6-/- hepatocytes. Similarly, AnxA6-/- mice showed low blood glucose levels during fasting due to altered alanine-dependent GNG. Mechanistically, cytoplasmic SNAT4 failed to translocate to the sinusoidal plasma membrane of AnxA6-/- hepatocytes after PHx, diminishing their ability to produce glucose from alanine. We conclude that lack of AnxA6 compromises alanine-dependent GNG and liver regeneration in mice.

Keywords: Alanine, gluconeogenesis, SNAT transporters, glucose metabolism, liver regeneration.

Phosphorylation of Annexin A2 is essential for its association with exosomes in triple negative breast cancer and for imparting proliferative and invasive phenotype to other cells

Priyanka Prakash Desai
University of North Texas, Denton, USA

Triple negative breast cancer (TNBC) accounts for 15-20 % of all the breast cancer cases. Studying TNBC is the utmost importance as treatment lacks targeted based therapies. Elevated levels of AnnexinA2 (AnxA2), a Ca+2-dependent phospholipid binding protein, has been correlated with worse overall survival in TNBC patients. Our previous data implicate that exosomal AnxA2 is involved in creating a pre-metastatic niche and facilitating metastasis in TNBC. Moreover, N-terminal phosphorylation of AnxA2 in cells at Tyrosine (Tyr) 23 has been implicated in regulating several AnxA2 activities in cancer progression. However, the mechanism for the association of AnxA2 with exosomes in TNBC cells has been least elucidated. Here, we demonstrated that N-terminal phosphorylation of AnxA2 at Tyr 23 is important for its association with exosomes and these exosomes impart invasive and proliferative phenotype in other cells. Hence, studying the above hypothesis may lead us closer to use AnxA2 as a therapeutic, diagnostic or prognostic marker in triple negative breast cancer.
N-terminal phosphorylation of AnxA2 at Tyr23 in the MDA-MB-231 TNBC cells (Phosphomimetic mutant cells) plays an important role in imparting invasive and proliferative phenotype to the TNBC cells. Phosphorylation of AnxA2 at Tyr23 plays a vital role in its association with the exosomal surface in TNBC. Moreover, phosphomimetic cell derived exosomal AnxA2 also increases invasive and proliferative capacity of other breast cancer cells. The above study will help to determine whether Exo-AnxA2-Y23EGFP facilitate formation of pre-metastatic niche and angiogenesis in distant organs, in-vivo, in triple negative breast cancer.

The project was funded by National Institute of Health's R01CA220273 to Dr. J.K.Vishwanatha.

Annexins in Health and Disease I

Session 3: Diseases (Cancer and Inflammation)

The Role of Annexin A1 in Resolving Thrombo-inflammation

Felicity N. E. Gavins[1,2], Elena Senchenkova[2], Junaid Ansari[2], Shantel Vital[2]
[1]Department of Life Sciences, Brunel University London, Uxbridge, UB8 3PH, UK.
[2]Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences CenterShreveport, Shreveport, Louisiana, USA.

Thrombosis and inflammation are intertwined processes which are major pathophysiological contributors of ischemia reperfusion injury (I/RI). The incidence of I/RI is far reaching, including stroke, myocardial infarction, and organ transplantation. Therefore, the administration of agents that resolve the aggressive thrombo-inflammatory state effectively after ischaemic insults represent attractive novel treatment strategies for I/RI.

Resolution is an active process involving a tightly orchestrated series of highly regulated biochemical mediators, including the endogenous protein Annexin A1 (AnxA1). Although much work has focused on the effect of AnxA1 on leukocyte accumulation during I/RI, less is known regarding its effect on platelets and the therapeutic potential of AnxA1 in resolving thrombo-inflammation after I/RI. Using in-vitro, ¬in-vivo, genetic and pharmacological approaches, coupled with sophisticated imaging platforms and clinical samples, we are in the process of closing this knowledge gap to discern the effect(s) of AnxA1 on platelets to determine whether strategies focused on the AnxA1-Fpr2/ALX axis may substantially add to the development of new treatment possibilities for I/RI.

Funds supporting this research have been received from the Royal Society Wolfson Foundation (RSWF\R3\183001) and the National Institutes of Health/National Heart, Lung, Blood Institute (1R01HL134959).

Annexin A2 Integrates Fibrinolysis with Angiogenesis and Retinal Injury

Katherine Amberson Hajjar
Weil Cornell Medical College, New York, USA

Annexin A2 (A2) is a ubiquitous, multifunctional protein that forms the heterotetrameric (A2•S100A10)2 complex, which binds fibrinolytic proteins and accelerates activation plasmin on cell surfaces. Anxa2-/- mice display microvascular fibrin accumulation and have a prothrombotic response to vascular injury. Humans with high titer anti-A2 antibodies have clinical thrombosis, and excessive expression of A2 is associated with bleeding. Anxa2-/- mice are resistant to hypoxia-induced retinal neovascularization, a model of diabetic retinopathy. We have now observed that A2 promotes the response to penetrating ocular injury known as proliferative vitreoretinopathy (PVR), which affects over 200,000 individuals worldwide each year. In PVR, retinal pigment epithelial (RPE) cells migrate from beneath the retina, to the retina's vitreal surface, where they form epiretinal membranes, which can contract causing retinal detachment and severe loss of vision. In the dispase-induced model, Anxa2-/- mice are resistant to PVR, showing minimal RPE cell migration and only slight epiretinal membrane formation. Macrophage-induced migration of RPE cells requires A2-related cell surface fibrinolytic activity, and can be blocked by anti-A2 IgG. Thus, A2 supports two host responses, hypoxia-induced angiogenesis and the PVR response to retinal injury. In both, cell migration is dependent upon cell surface fibrinolytic activity that is supported by A2.

The role of annexin a1/fpr2 axis in pneumococcal pneumonia and polymicrobial sepsis

Lirlandia Pirez de Sousa
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AnnexinA1 (AnxA1) is a pro-resolving protein that mainly acts through the Formyl Peptide Receptor (FPR)-2/ALXR modulating inflammation in several disease models. Pro-resolving based therapies, rather than anti-inflammatory drugs, decrease inflammation without causing immunosuppression. Of note, studies that address the specific role of pro-resolving mediators, such as AnxA1, during infections are still incipient. Our group has focused on the study of AnxA1 in two relevant experimental models of infectious diseases: pneumococcal pneumonia and sepsis (cecal ligation and puncture - CLP). Our findings showed that AnxA1 and Fpr2/3 deficient (KO) mice were highly susceptible to pneumococcal infection, displaying uncontrolled inflammation, increased bacterial burden and pulmonary dysfunction. Importantly, treatment of WT, but not Fpr2/3 KO, infected mice with Ac2-26 decreased inflammation, lung damage and bacterial burden improving macrophage phagocytosis, pointing out that AnxA1 via FPR2 promotes host defenses while controlling inflammation and bacteria dissemination during pneumonia. In sharp contrast, AnxA1-/- mice showed an unexpected protection during CLP, a result that could not be attributed to the FPR2 actions, since, as expected, Fpr2/3 KO mice were more susceptible to CLP. During sepsis, AnxA1-/- mice enhanced early host defenses improving bacterial control and systemic inflammation. The complex dynamics of the inflammatory response to different infectious diseases may explain the contrasting outcomes and uncover the need to understand the interplay between pathogens and the host defenses coordinated by AnxA1.

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Uterine receptivity is increased by AnxA1 via FPR and ERK

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Annexin A1 (AnxA1) is a key pro-resolution protein, ligand of formyl peptide receptors 1 (FPR1) and/or 2 (FPR2). We have previously shown that AnxA1 controls in vivo reproduction by modulating inflammation in the microenvironment of uterine implantation of blastocyst, especially by enhancing interleukin-6 (IL-6). Hence we here investigated the mechanisms of AnxA1 actions on the effect. AnxA1 (1,35nM) increased Be Wo spheroid attachment on uterine epithelial cells (Ishikawa cells; IK), which was inhibited by co-incubation with Boc-2, a pan FPR inhibitor. AnxA1 increased the expression of molecules in IK involved on uterine receptivity and embryo implantation, such as Mucin-1 (MUC-1), Claudin-1 and Zona occludens-1, and the effects were abrogated by co-incubation with Boc-2 (5μM) or WRW4 (10 μM), a FPR2 antagonist. The enhanced MUC-1 expression was not due elevated IL-6 secretion, as AnxA1 reduced IL-6 production by IK. The effect of AnxA1 on MUC-1 expression occurred via MAP Kinase activation but not via NF-□B and STAT-1□, as only treatment with ERK inhibitor impaired AnxA1-induced MUC-1 expression. AnxA1 is up-expressed in the uterine sites of implantation in vivo. In summary, here we show AnxA1 controls embryo implantation via FPR activation, modulating key molecules in the uterine epithelium. MAPK/ERK is a crucial pathway for the control of MUC-1 exerted by AnxA1. Therefore, AnxA1/FPR1/MAPK axis emerges as a potential tool for fertility management.

Keywords: BeWo spheroid, mucin-1, embryo implantation, MAP kinase, Ishikawa cells

Diverse roles of annexin A6 in triple negative breast cancer

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Annexin A6 (AnxA6) is implicated in several membrane-associated processes and in a wide range of cellular functions including cell growth and motility as a membrane and Ca2+ binding protein. AnxA6 has also been shown to be involved in the regulation of cholesterol homeostasis, vascular calcification, Ca2+ influx into cells, membrane repair, and GTP binding. However, the involvement of AnxA6 in these clearly diverse cellular functions especially in triple negative breast cancer remains intriguing. To fill this knowledge gap, we have shown that AnxA6 is a tumor suppressor in breast cancer; influences tumor cell adhesion/spreading via regulation of focal adhesions; promotes cell motility via modulation of Ca2+ activated RasGRF2-mediated inhibition of small GTPases such as Cdc42; modulates tumor cell growth via the Ca2+ activated RasGRF2 and Ras proteins; and promotes resistance to EGFR-targeted therapies via stabilization of activated EGFR on the cell surface and accumulation of cholesterol in late endosomes. Finally, reduced expression of AnxA6 is more relevant in TNBC molecular subtypes and following neoadjuvant chemotherapy and that AnxA6 may be an independent predictor for distant relapse-free survival and a potential biomarker to discriminate responders from non-responders of chemotherapy treated TNBC patients. Taken together, AnxA6 plays an important role in breast cancer progression, resistance to therapy and may be a reliable biomarker for response of TNBC to therapy.

Keywords: Annexin A6; Breast cancer; EGFR; RasGRF2; Chemotherapy

Annexins in Health and Disease I

Session 4: Diagnosis and treatment

Annexin A1 and the control of joint disease: local and distant effects

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While an integrated inflammatory response – with tight temporal and spatial control - is crucial for a physiological well-being status, a loss of regulation of these mechanisms can lead to fibrosis and/or chronic diseases [1]. Over the last two decades we and others have demonstrated that the second phase of the acute inflammatory response, which we now call the resolution phase, is an active process, mediated by specific mediators and receptors. Resolution has been defined by a series of specific cellular responses like apoptosis and efferocytosis, with a main focus on the relevance of cells of the immune system like macrophages and granulocytes. Annexin A1 (AnxA1) is a major player in modulating the host response to inflammation and infection. As such, work over the years has identified several bioactions for AnxA1 that makes it a non-redundant mediator of resolution in several experimental settings.In the context of experimental arthritis, AnxA1 displays subtle effects with some modulatory function on the protective effects of glucocorticoids, but not major protection on joint disease when given directly along a pharmacological approach [2]. In other settings, though, direct intra-articular injection of AnxA1 and AnxA1+ vesicles exhibit tissue protective actions on cartilage integrity [3]. Our focus now is to establish the potential for AnxA1 to modulate comorbidities associated with arthritis and address the clinical observation that RA patients have double incidence of cardiomyopathy. On-going work on the potential for AnxA1 to control this secondary organ injury will be presented.Funds supporting this research have been received from the Medical Research Council UK (project MR/K013068/1), Wellcome Trust (programme 086867), William Harvey Research Limited.

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Annexins as biomarkers

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Annexins constitute a family of intriguing proteins. They can participate in a myriad of intra- and extracellular processes. In addition, annexins are appreciated as biomarkers, meaning that they indicate biological processes, physiological as well as pathophysiological ones, and reflect responses to an exposure or intervention.
Annexin A5 (AnxA5) is well-known for its biomarker potential in preclinical and clinical research. It was discovered as anticoagulant and evolved into a valuable biomarker indicating apoptosis in vitro and in vivo in animal models and in patients. AnxA5's biomarker potency relies on its ability to bind phosphatidylserine (PS), a phospholipid species the topology of which is tightly regulated in the plasma membrane (PM). Immuno-inflammatory stimuli and ageing can trigger translocation of PS from the inner to the outer leaflet of the PM. PS in the outer leaflet of the PM acts as a danger associated molecular pattern (DAMP) triggering clearance of the cell by phagocytosis.
PS exposing cells can be visualized using anxA5 which is coupled to a reporter, for example a fluorophore or a radionuclide. PS is a ubiquitous DAMP. All cell types, including the anuclear erythrocyte and platelet, can respond to stimuli by PS exposure. Recently, it was found that anxA5 also binds subsets of extracellular vesicles exposing PS.
This omnipresence of PS exposure broadens the applications of the biomarker anxA5 but, concurrently, also limits its diagnostic precision when used as a stand-alone biomarker.

Dectin-1 binding to annexins on apoptotic cells induces peripheral tolerance via NADPH oxidase-2

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Uptake of apoptotic cells by dendritic cells (DCs) and induction of a tolerogenic DC-phenotype is an important mechanism for establishment of peripheral tolerance to self-antigens. The receptors involved and underlying signaling pathways are not fully understood. Here, we identify Dectin-1 as a crucial tolerogenic receptor binding with nanomolar affinity to the core domain of several annexins (annexin A1, A5 and A13) exposed on apoptotic cells. Annexins bind to Dectin-1 on a site distinct from the interaction site of pathogen-derived β-glucans. Subsequent tolerogenic signaling induces selective phosphorylation of the spleen tyrosine kinase (SYK), causing activation of NADPH oxidase-2 and moderate production of reactive oxygen species. Thus, mice deficient for Dectin-1 develop autoimmune pathologies (autoantibodies and splenomegaly) and generate stronger immune responses (cytotoxic T-cells) against apoptotic cells. Our data describe a new immunological checkpoint system and provide a novel link between immunosuppressive signals of apoptotic cells and maintenance of peripheral immune tolerance.

Keywords: Dectin-1, apoptotic cells, peripheral immune tolerance, spleen tyrosine kinase (SYK), NADPH oxidase-2 (NOX-2).

The destiny of dying retinal ganglion cells in glaucoma

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Glaucoma is a neurodegenerative disease in which various triggers induce cascades of secondary events, which ultimately lead to apoptotic retinal ganglion cell (RGC) death. Apoptosis is generally considered to be irreversible, especially at late times when cell death-executing activities occur. However, recent studies reveal that recovery of dying cells is possible, even after reaching critical cell death events This phenomenon is termed anastasis. Promoting anastasis may represent a previously unrecognized beneficial mechanism of preserving differentiated cells that are difficult to replace, such as retinal ganglion cells.
In this study, differentiated PC12 cells were used as model for RGCs. We triggered these cells to undergo apoptosis with staurosporine and recorded their dynamics and morphology using live cell imaging: We observed dynamic local changes in individual cells during degeneration in vitro. Apoptotic PC12 cells showed chromatin condensation, nuclear fragmentation, plasma membrane blebbing, cell shrinkage, cell surface exposure of phosphatidylserine (PS), and formation of apoptotic bodies. Furthermore, we observed the progressive movement of apoptotic PS exposure along the axon towards the cell body, but in some cells PS exposure started at the cell body and then moved towards the axon. The results indicate that axons may not always be the first part of injury during neuron degeneration. On the basis of these data, we plan to study activation of caspase and release of mitochondrial cytochrome c in single neurons to investigate the mechanisms and the relationship between axon degeneration and soma, in order to gather more information for later dying cell recovery research.

Keywords: apoptosis; anastasis; annexin A5; live cell imaging

ANNEXINS 2019

Talks

Annexins in Health and Disease II

Session 5: Membrane Repair

Extracellular Role of Annexin-A2 in Muscle Repair and Degeneration

Marshall W. Hogarth[1], Aurelia Defour[1], Christopher Lazarski[1], Eduard Gallardo[2], Jordi Diaz-Manera[3], Terence A. Partridge[1,4], Kanneboyina Nagaraju[1,4] and Jyoti Jaiswal[1,4].
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Recovery from cell injury involves repair of injured plasma membrane which requires Annexin family of proteins. If the injured cell fails to repair the reparative process switches to tissue repair, which involves a coordinated set of interactions between the inflammatory and stem cells to clear the damaged tissue and regenerate the lost cells. One such stem cell type that helps regenerate injured muscles is the muscle-resident mesenchymal stem cells called the fibroadipogenic precursors (FAPs). But chronic muscle inflammation causes them to accumulate and adopt fibrotic or adipogenic fate leading to muscle damage. Muscle damage due to mutations in the plasma membrane repair protein dysferlin causes limb girdle muscular dystrophy 2B (LGMD2B). These muscles are characterized by chronic muscle inflammation and adipogenic replacement. We show that the adipogenic conversion of dysferlinopathic muscle is mediated by the action of extracellular Annexin A2 on inflammatory cells and FAPs. Extracellular Annexin A2 increases FAP accumulation and adipogenic differentiation, which can be prevented by Annexin-A2 knockout. Generation of Annexin A2 and Dysferlin double KO mice identified that lack of Annexin-A2 markedly reduces inflammation and FAP accumulation, identifying Annexin-A2 as the link between myofiber injury, inflammation, FAP proliferation and adipogenic muscle loss. These results show that disease initiation in dysferlinopathy depends on the extracellular Annexin A2 such that in the first step, poor muscle repair results in excess inflammation early in life. Later on, Annexin A2 facilitates fatty differentiation of FAPs causing muscle wasting and rapid worsening of the clinical symptoms. This provides a novel mechanistic role of Annexin A2 in tissue repair and identifies downregulation of Annexin A2 as a novel avenue to intervene in the onset and progression of adipogenic muscle loss.

Mechanisms of annexin mediated plasma membrane repair

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University of Copenhagen, Denmark

The plasma membrane of eukaryotic cells defines the essential boundary to the extracellular environment and, thus injuries to the cell membrane pose a lethal threat to cells. Cells cope by activating their plasma membrane repair system, which includes mechanisms to reseal and remove damaged membrane. Annexin proteins are involved in various steps of the plasma membrane repair system. Our recent studies show that they serve more distinct functions in the repair response than previously assumed by regulating local membrane curvature and curvature sensing. Cancer cells are more dependent on efficient plasma membrane repair to counteract stress-induced membrane injuries, which opens novel avenues to target cancer cells through their repair system. To target annexin-mediated repair in cancer cells we have elucidated the effect of the compound trifluoperazine (TFP) as a potential pan-annexin inhibitor. We find that TFP sensitizes cancer cells to various plasma membrane injuries without restricting annexin translocation or binding to the injured membrane. Biophysical approaches using supported membrane model revealed that TFP inhibits curvature-responses triggered by annexin A2, A4 and A5 probably by directly changing the fluidity of the membrane. Thus, TFP or phenothiazines in general may provide a novel avenue for cancer treatment by compromising membrane repair. Here, novel aspects of plasma membrane repair and regeneration implicating annexins will be discussed.

Keywords: Plasma membrane repair; Regeneration; Annexins; Trifluoperazine; Cancer.

Dysferlin-mediated phosphatidylserine sorting engages macrophages in sarcolemma repair

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Failure to repair the sarcolemma leads to muscle cell death, depletion of stem cells and myopathy. Hence, membrane lesions are instantly sealed by a repair patch consisting of lipids and proteins such as Annexins and Dysferlin. It has remained elusive how this patch is removed to restore cell membrane integrity. We examine sarcolemmal repair in live zebrafish embryos by real-time imaging. Here various Annexins show a sequentially orchestrated accumulation, while on EM resolution they represent an amorphous mass at the lesion site. Moreover, macrophages remove the patch. Phosphatidylserine (PS), an “eat-me” signal for macrophages, is rapidly sorted from adjacent sarcolemma to the repair patch in a Dysferlin (Dysf) dependent process in zebrafish and human cells. A previously unrecognized arginine-rich motif in Dysf is crucial for PS accumulation. It carries mutations in patients presenting with limb girdle muscular dystrophy 2B. This underscores the relevance of this sequence and uncovers a novel pathophysiological mechanism underlying this class of myopathies. Our data show that membrane repair is a multi-tiered process involving immediate, cell-intrinsic mechanisms as well as myofiber/macrophage interactions.

Keywords: membrane repair, dysferlin, annexins, phosphatidylserine, macrophage, zebrafish

Down-regulation of Annexin A1 in human placenta may be responsible for the development of preeclampsia

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Preeclampsia (PE) is a major hypertensive human disease affecting up to 8% of pregnancies, which is one of the main identified causes of severe premature births. There is currently no curative or preventive treatment for PE. The human placental trophoblast, which is an epithelium-like tissue covering the placenta, plays the role of a barrier separating maternal and fetal blood. Disturbances of trophoblast functions are observed in pathological conditions such as PE and are implicated in abnormal fetal growth and development. The transcription factor STOX1 (Storkhead Box1) has been discovered through the association of genetic polymorphisms located inside the ORF of the gene with familial forms of PE.

We have shown that human STOX1-overexpressing trophoblast BeWo cell line presents a massive down-regulation of Annexin A1, a defect in membrane repair and a subsequent cell death. We hypothesize that PE is a multifactorial disease to which defect of the membrane repair machinery in trophoblasts may contribute. The absence of Annexin A1 may lead to the death of membrane-damaged trophoblasts by necrosis. Necrotic cells and extracellular vesicles that are released in the maternal blood carry signaling molecules that may be responsible for inducing maternal arterial hypertension and inflammation, which are hallmarks of PE.

Keywords: membrane repair, Annexin A1, preeclampsia, placenta

Annexin A6 in membrane repair of human skeletal muscle cells

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Plasma membrane disruption is a physiological event occurring in cells exposed to mechanical stress, such as skeletal or cardiac myocytes, epithelial cells and endothelial cells. Whereas normal muscle cells are able to reseal membrane ruptures rapidly, defect in membrane repair contributes to the development of limb girdle muscular dystrophy type 2B and Miyoshi myopathy. In addition, in search for genetic modifiers in muscular dystrophy, McNally and coworkers have identified a splice site variant giving rise to a truncated Annexin-A6 (AnxA6) in dysferlin-null mice, which compromises AnxA6 translocation to membrane wound.

We have developed methodologies enabling to unravel membrane repair mechanisms in human skeletal muscle cells and identify the components of the resealing machinery, focusing on the protein family called the Annexins (Anx).

Here, we study the involvement of AnxA6 in membrane repair of human skeletal muscle cells. Using myotubes expressing Anx coupled with a fluorescent protein (notably GFP) and laser irradiation to damage their plasma membrane, we demonstrate that AnxA6 is recruited at the disruption site within seconds after plasma membrane injury, and forms a “cap” as observed in murine skeletal muscle. In addition, Anx subcellular localization at the disruption site can be observed at high resolution using correlative light and electron microscopy. Our results indicate that AnxA6 seems to be associated with membranes of the damaged area.

Keywords: membrane repair, Annexin-A6, correlative light and electron microscopy, skeletal muscle

Annexins in Health and Disease I

Session 6: Host-pathogen interactions

The annexins: evidence tape for membrane lesions

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University of Bern, Switzerland

Plasma membrane repair strategies are dependent on the nature of the injury and on the type of the affected cell. However, their common characteristic is their dependence on intracellular Ca2+ elevation. A breach in membrane integrity followed by the influx of extracellular Ca2+ will lead to the translocation of members of the annexin protein family to the affected site. Since each annexin has a distinct threshold of Ca2+-dependent membrane binding, plasmalemmal repair depends on the intracellular Ca2+ gradient and on the amount and availability of suitable annexins for the type of repair that is required. Acute, spatially confined membrane lesions are predominantly eliminated by active outward vesiculation and vesicle shedding. Chronic membrane stress is characterised by a sustained upregulation of annexins, which points to an active setting of cellular Ca2+ tolerance limits to fit individual physiological requirements.
Since annexins have been described to interact with other molecules, which participate in membrane repair they must be considered protective all-rounders.

Heterotetrameric annexin A2/S100A10 (A2t) is essential for oncogenic human papillomavirus trafficking and capsid disassembly, and protects virions from lysosomal degradation.

Martin Kast
University of Southern California, Los Angeles, USA

Human papillomavirus (HPV) entry into epithelial cells is independent of canonical endocytic pathways. Upon interaction with host cells, HPV establishes infection by traversing through an endocytic pathway that is clathrin- and caveolin-independent, but dependent on the annexin A2/S100A10 heterotetramer (A2t). We examined the contribution of monomeric annexin A2 (AnxA2) vs. A2t in HPV infection and endocytosis, and further characterized the role of these molecules in protein trafficking. We specifically show that cell surface A2t is not required for HPV attachment, and in the absence of A2t virion internalization remains clathrin-independent. Without A2t, viral progression from early endosomes to multivesicular endosomes is significantly inhibited, capsid uncoating is dramatically reduced, and lysosomal degradation of HPV is accelerated. Furthermore, we present evidence that AnxA2 forms a complex with CD63, a known mediator of HPV trafficking. Overall, the observed reduction in infection is less significant in the absence of S100A10 alone compared to full A2t, supporting an independent role for monomeric AnxA2. More broadly, we show that successful infection by multiple oncogenic HPV types is dependent on A2t. These findings suggest that A2t is a central mediator of high-risk HPV intracellular trafficking post-entry and pre-viral uncoating.

Annexin A3: An Important Host Factor for HCV Infection

Hanna Bley
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The hepatitis C virus (HCV) life cycle is tightly connected to the host lipid metabolism with lipid droplets (LDs) serving as an assembly site for HCV. Quantitative lipid droplet proteome analysis of HCV-infected cells identified annexin A3 (ANXA3) as an important host factor for HCV maturation and egress. In HCV-infected cells ANXA3 is recruited to lipid-rich fractions by HCV-core and NS5A. Whereas ANXA3 knockdown does not impact HCV replication, it is essential for the interaction of the viral envelope protein E2 with the apolipoprotein (Apo) E and for the relocalization of ApoE in HCV-infected cells. However, a direct interaction between ANXA3 and viral particles or ApoE was not detected and the molecular details of ANXA3 recruitment to LDs remain unclear. Most likely adaptor proteins mediate the binding and re-routing of ApoE as well as the trafficking of ANXA3 to lipid droplets.
To further characterize molecular details of HCV assembly, maturation, and egress and to identify interaction partners of ANXA3, we used the recently described proximity labeling methods biotin identification BioID2 as well as the engineered ascorbate peroxidase APEX2. BioID2 and APEX2 were genetically fused to ANXA3 to explore interacting proteins during HCV infection. Therefore, HCV-infected and uninfected cells stably expressing the fusion proteins were prepared for SILAC labeling. Biotinylated proteins identified by mass spectrometry were investigated for their possible roles in the HCV life cycle. Thus, both methods will help to decipher ANXA3-interacting proteins and therefore enable us to study the molecular mechanisms of the HCV life cycle in more detail.

Keywords: Annexin A3, Hepatitis C Virus, Lipid Droplets, Virus Morphogenesis

Cellular survival following membrane perforation by bacterial pore-forming toxins relies on microvesicle-shedding efficiency

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University of Bern, Switzerland

Bacterial infectious diseases can lead to death or serious illnesses. These outcomes are partly the consequence of pore-forming toxins, which are secreted by pathogenic bacteria (e.g. pneumolysin (PLY) of Streptococcus pneumoniae). PLY binds to cholesterol in the plasma membrane and assembles to form trans-membrane pores, which can lead to Ca2+ influx and cell death. A rise of cytoplasmic Ca2+ levels recruits annexins to the plasma membrane, where they cross-link adjacent membranes to plug the toxin pore and assist in the removal of injured membrane areas by microvesicle-shedding.
Immune cells fight bacterial infections and thus critically rely on membrane repair after PLY attacks. We investigated the susceptibility of different immune cells, e.g. lymphoid T-cell line Jurkat and myeloid cells (U937, THP-1), to PLY. We show that Jurkat T-cells are highly susceptible, whereas myeloid cells are less susceptible to PLY. We find that Jurkat T-cells express lower levels of annexins, e.g. annexin A2, as compared to myeloid cells. We show that the differences in PLY-susceptibility in different immune cells are mostly dependent on the capability of Ca2+ induced microvesicle-shedding. Moreover, microvesicle-shedding is facilitated by overexpression of annexin A2 and enhances cell survival after PLY attack. Our results suggest that myeloid cells strongly benefit from an efficient cellular repair machinery whereas in lymphoid cells these repair mechanisms are poorly operating.

Keywords: bacterial pore-forming toxins, immune cells, host-defense, plasma membrane repair, annexin

Annexin A8 is a major factor in dendritic cell maturation and functionality

Sebastian Schloer
University of Muenster, Germany

The immune system is well balanced and composed of the innate and adaptive immunity. The interface between both components, innate and adaptive immunity, is occasional formed by antigen presenting cells e.g. dendritic cells (DCs) which are programmed to recognize pathogenic structures while they do not react to self-substances. These two components of the immune system create a dynamic biological environment where particular DCs decide if the immune system is alert to pathogens or not. Pathogen associated molecular patterns (PAMPs) e.g. lipopolysaccharide (LPS) or Lipoteichoic acid (LTA) are registered through specific receptors, called pattern recognition receptors (PRRs) like Toll-like receptor 2 and 4 (TLR). The LPS-TLR4 signaling axis triggers the maturation of DCs by activating several transcription factors like nuclear factor κ-light chain enhancer of activated B cells (NFκB) or signal transducers and activators of transcription 3 and 5 (STAT). The transcription factors are phosphorylated and then translocated to the nucleus where they drive the expression of a variety of cytokines that are secreted to modulate the course of the immune response. Here, we demonstrated that annexin A8 (AnxA8), a member of the highly conserved annexin protein superfamily, is crucial for the maturation process of DCs. The knockout of AnxA8 (AnxA8KO) impaired the number of mature DCs (mDCs) and their functionality as the phagocytosis competence and the secreted cytokine levels were significantly reduced. We further monitored the activation of several transcription factors like NFκB which was markedly affected in AnxA8KO DCs. Finally, we also investigated that AnxA8 and AnxA3 expression is regulated during DC maturation facilitating the maturation process.

Keywords: annexin A8, cholesterol, dendritic cells, electron microscopy, lipid rafts, toll-like receptor

Annexins Latest Developments and Future Perspectives

Session 7: Diseases (Cancer and Inflammation)

Annexin-A1- friend or foe?

Lina Lim Hsiu Kim
National University of Singapore

Annexin A5 in vascular diseases

Anna Frostegard
Karolinska Instituet, Solna, Sweden

Two Arabidopsis annexins function in transporting sugar to root tips to affect primary root growth

Greg Clark
University of Texas, Austin, USA

Annexins are a multigene, multifunctional family of calcium-dependent membrane-binding proteins that play important roles in plant cell signaling. In the plant model system, Arabidopsis, two annexins, Ann1 and Ann2, are 64% identical at the amino acid level, and are both highly expressed in seedlings. Here, we show that ann1 and ann2 loss-of-function mutant seedlings grown in the absence of sugar show decreased primary root growth and modified root cap structures, but these mutant defects are rescued by sucrose, glucose, or fructose. In seedlings grown without sugar, significant up-regulation of photosynthetic gene expression and chlorophyll accumulation occurs in ann mutant seed leaves compared to wild type, which indicates potential sugar starvation in the roots of ann mutant seedlings. Unexpectedly, the overall sugar content of ann mutant primary roots is significantly higher than that of wild-type roots when grown without sugar. To examine the transport of sugar in roots to the root tip, we observed the unloading pattern of carboxyfluorescein dye and found that post-phloem sugar transport was impaired in ann mutant root tips compared to wild type. In the mutant root tips we also detected increased levels of ROS and callose, the latter of which would restrict plasmodesmal sugar transport to root tips. Our results indicate that Ann1 and Ann2 play important roles in post-phloem sugar transport to the root tip, which in turn influences photosynthesis in leaves.

Keywords: plant, growth, genetic mutant, transport phenotype, ROS.

Annexin A2 binds to the internal ribosomal entry site of c-myc mRNA in the presence of calcium to inhibit translation

Anni Vedeler
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Annexin A2 (AnxA2) is part of mRNP complexes containing c-myc mRNA and other specific mRNAs translated on cytoskeleton-bound polysomes. We have previously identified an AnxA2-binding site in the localisation signal of the 3'-untranslated region (UTR) of the c-myc mRNA , which is responsible for its the perinuclear localisation. Furthermore, alignment studies and UV-crosslinking experiments lead us to suggest that a five nucleotide (nt) consensus sequence, 5'-AA(C/G)(A/U)G, together with higher order structures of the c-myc and anxA2 3'-UTRs, are involved in the interaction between c-myc mRNA and AnxA2. We have now identified AnxA2 as a c-myc internal ribosomal entry site (IRES)-binding protein and determined the mRNA binding site as the region encompassing nt 195-330 of the mouse c-myc 5'UTR. This region also contains the AAGAG consensus sequence found in the c-myc 3'UTR. Deletion of the AnxA2-binding region (nt 195-330) totally abolished the inhibition by AnxA2 of in vitro translation of the chimeric c-myc mRNA reporter, indicating that the protein may interact with canonical factors associating with the ribosomal docking site at the IRES. For example, it has been shown that AnxA2 interacts with the receptor for activated C kinase 1 (RACK1), a ribosome-associated protein implicated in the regulation of IRES activity. These findings suggest that AnxA2 acts as a switch to turn off the c-myc IRES activity in the presence of calcium; possibly during c-myc mRNA transport.

Keywords: Annexin A2, c-myc mRNA, IRES, translation

Gonadotropin releasing hormone (GnRH) regulation of ANXA5 containing bleb and microvesicle formation in the pituitary gonadotropes

Mitsumori Kawaminami
University of Science, Okoyama, Japan

We have demonstrated that gonadotropin releasing hormone (GnRH) stimulates the expression of annexin A5 (ANXA5) in the pituitary gonadotropes, and ANXA5 augments gonadotropin secretion and enhances GnRH stimulation of gonadotropin release. It is, however, still obscure how ANXA5 augments gonadotropin release at gonadotropes. Although extracellular ANXA5 was shown to stimulate gonadotropin release, it is also unknown how ANXA5 traverses plasma membrane without a signal sequence. We studied first in the present study that changes in the distribution of ANXA5 in the gonadotrope after GnRH stimulation and found blebs containing ANXA5 were formed on LβT2 mouse gonadotropes shortely after GnRH stimulation. Double immunocytochemistry for ANXA5 and LHβ showed bleb formation also occured on gonadotropes in the primary culture of rat pituitary cells. The conditioned medium of GnRH stimulated LβT2 was sequentially centrifuged at 20,000 xg and 110,000 xg to obtain microvesicle/ectosome and exosome respectively. Microvesicle containing ANXA5 was increased from 10 to 180 minutes after GnRH stimulation. Microvesicle is a pinched off vesicle of extruded membrane. Size is more than exosome (more than 100 nm) and separated by centrifugation at 20,000 xg. GnRH agonist treated microvesicle fraction significantly stimulated LH release in a dose dependent manner in LβT2 culture. The blebbing induced by GnRH agonist was inhibited by H89, protein kinase A inhibitor. It is suggested that Gαs signaling mediates GnRH stimulation of blebbing and microvesicle formation. Present study first demonstrates a hormonal regulation of microvesicle formation and it is the auto-enhancing mechanism of LH secretion.

Keywords: annexin A5, GnRH, microvesicle, pituitary, gonadotrope.

Poster Abstracts

Anxa5 protects hyper-mineralization at the enthesis by differential expression of pyrophosphate regulators

Akira Nifuji

We have previously shown that annexin a5 (Anxa5) regulates bone overgrowth formation in adult mice. In Anxa5-deficient (Anxa5-/-) mice, bone overgrowth at the enthesis was observed, which was mediated through mechanical forces brought by muscle loading. In this study, we investigated molecular mechanisms how Anxa5 regulates bone overgrowth. Expression of a positive regulator of mineralization, tissue non-specific alkaline phosphatase (TNAP), was increased at the enthesis in Anxa5-/- mice, whereas expressions of two negative regulators, ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) and the progressive ankylosis (Ank) , were decreased in vivo. In tenocytes and chondrocytes which constitute entheses, siAnxa5 transfection (Anxa5 KD) resulted in increase in TNAP mRNA expression. In contrast, it decreased expressions of ENPP1 and Ank mRNA. We further measured extracellular inorganic pyrophosphates (ePPi) / inorganic phosphates (ePi) levels in vitro and found that ePPi was decreased whereas ePi was increased in Anxa5 KD tenocytes. In addition, ENPP1 and Ank mRNA expressions were increased in cells where extension force was applied in vitro, however, they were inhibited in Anxa5 KD tenocytes even in the application of the extension force. Taken together, Anxa5 protects hyper-mineralization at the enthesis by differential expression of pyrophosphate regulators.

Binding of Annexin A2 membranes containing novel cholesterol analogue

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Lipid microdomains regulate cellular processes by serving as organizing centers, affecting the assembly of signaling molecules and diverse proteins. The dynamic organization of the domains is driven by intrinsic properties of the membrane lipids, as well as integral or/and associated proteins. Of importance, associated proteins can specifically interact with certain membrane lipids and affect the distribution. The annexin family comprises such membrane-binding proteins that can induce the formation of cholesterol and PI(4,5)P2 rich membrane microdomains in artificial membrane system and also in cellular membranes in a Ca2+ dependent manner. A quantitative analysis of the membrane binding parameters of annexin A2 (AnxA2) was performed by employing the Quartz Crystal Microbalance with dissipation. The AnxA2 membrane binding, is characterized by a positive cooperativity that requires the presence of cholesterol and is mediated by the conserved C-terminal annexin core domain. Here, we exploited this AnxA2 property to characterize novel cholesterol-based imidazolium salts that incorporate into artificial and in cellular membranes and behave comparable to endogenous cholesterol. Importantly, the cellular distribution of the cholesterol analogues can be monitored by live cell imaging experiments. We performed a series of AnxA2 membrane binding experiments and assessed whether the novel cholesterol analogue can replace endogenous cholesterol in regulating the mode of AnxA2-membrane interactions. Results indicate that the novel cholesterol analogues do not affect AnxA2 binding and can be used to reveal the dynamic distribution of cholesterol in live cells.

Keywords: Annexin A2, Cholesterol, QCM.

Annexin A4 N-Terminus– a specific inhibitor of adenylyl cyclase type 5

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Question: Annexin A4 (A4) was identified as a negative regulator and interaction partner of Adenylyl Cyclase type 5 (AC5), which is a key element in cAMP-dependent signal transduction. The aim of the study was to investigate the A4 – AC interaction focusing on the main cardiac isoforms AC5 and AC6.
Methods: Coimmunoprecipitation experiments, performed in HEK293 cells transiently transfected with wildtype A4 (A4 WT) or A4 lacking the N-terminus (A4 ΔN) and YFP-tagged AC5 or GFP as control, were used to delineate if the A4 N-terminus is critical for binding of AC5. The effect of A4 N-terminal peptide (A4N1-22) on cAMP level was tested in HEK293, co-transfected with AC5 or AC6 and for FRET measurements additionally with an EPAC-FRET sensor. After incubation with peptide and NKH477/IBMX time-dependent FRET-ratio change was detected by confocal microscopy and quantitative cAMP values were determined by ELISA.
Results: YFP-AC5 was precipitated and A4 WT could be detected in eluate and not A4 ΔN. A4N1-22 was able to reduce AC5- and not AC6-mediated cAMP-level under stimulated conditions determined by ELISA as well as confocal measurements.
Conclusion: We identified A4 N-terminal peptide as a specific inhibitor that can discriminate between AC5 and AC6. These results could provide the basis for developing a specific AC5-Inhibitor as a novel option for heart failure treatment.

Keywords: annexin A4, heart failure, adenylyl cyclase 5, cAMP.

Pharmacological treatment with annexin A1-derived peptide protects against cisplatin-induced hearing loss

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This study evaluated the therapeutic potential of the anti-inflammatory annexin A1 (AnxA1)-derived peptide, Ac2-26, on cisplatin-induced ototoxicity model. Wistar rats received intraperitoneal injection of cisplatin (10mg/kg for 3 days) for induction of hearing loss. Another group was treated with Ac2-26 (1mg/kg) 15 minutes before cisplatin administration. Control animals received saline. Hearing thresholds were measured by distortion product otoacoustic emissions (DPOAE) before and after treatments. Histological, immunohistochemical and ultrastructural analysis were performed after 6h of last cisplatin administration. Pharmacological treatment with Ac2-26 protects against cisplatin-induced hearing loss, evidenced by DPOAE that showed similar signal-noise ratio between control and Ac2-26-treated groups. These otoprotective effects of Ac2-26 were associated with the increased numbers of spiral ganglion neurons (7327±329 cells/mm2; $p<0.01$) compared to the non-treated cisplatin group (5818±261). Additionally, Ac2-26 produced lower immunoreactivity of cleaved caspase 3 in the neurons in relation of the cisplatin group ($p<0.001$). Similar results of Ac2-26 treatment were detected in the vestibular ganglion neurons, indicating a neuroprotective effect of this peptide. In conclusion, our results suggest that Ac2-26 has a substantial otoprotective effect in this cisplatin-induced ototoxicity model, evidencing AnxA1 as a potent therapeutic target in the pathophysiology of hearing loss.

Keywords: distortion product otoacoustic emissions, immunohistochemistry, inner ear, neuron, rat model.

Support: FAPESP (2017/26872-5); CAPES/PRINT (88881.310737/2018-01).

The role of annexin 1 from Arabidopsis thaliana in oxidative stress.

Dorota Konopka-Postupolska

Plant genomes encode up to a dozen annexins and at least some of them are involved in maintaining the redox poise in plant cells. Besides, annexin 1 from Arabidopsis thaliana (ANN1) was shown to alleviate oxidative stress also in heterologous cells. This strongly suggests that such an effect is due to the interference with very basic molecular mechanisms, common to different kingdoms of life. In plants, reactive oxygen species (ROS) metabolism provides a platform for interaction of internal metabolism with environmental factors. The specificity of stress stimulus is encoded in the profile of accumulated ROS. But the biological activities of defined molecular species of ROS differ and separate pathways can antagonize or enhance each other. For example, decreased accumulation of hydrogen peroxide (H2O2) results in increased activation of singlet oxygen (1O2-)-induced gene expression. To examine in detail the role of ANN1 in the stress response, we increased the level of ANN1 in Arabidopsis mutants that accumulate singlet oxygen or H2O2 in the chloroplast and analyzed the footprint of such modification on ROS-mediated pathways. The whole-genomes transcriptomes of modified and unmodified plant lines stress were done. Obtained results shed a light on the physiological functions of ANN1 in oxidative stress in plant cells and help to understand during high light the ANN1-mediated protection.
This work was supported by a grant from the National Science Centre 2015/19/B/NZ3/01476

Keywords: plant annexins, oxidative stress, stress, singlet oxygen, hydrogen peroxide

Annexin-coated particles induce antigen-specific immuno-suppression

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Apoptotic cells mediate the development of tolerogenic dendritic cells (DC) and thus facilitate the induction and maintenance of peripheral tolerance. Following the identification of the evolutionary conserved annexin core domain (Anx) as a specific signal on apoptotic cells which antagonizes Toll-like receptor (TLR) signaling, we examined whether the tolerogenic capacity of Anx can be exploited to downregulate antigen-specific immune responses. The treatment of bone marrowderived dendritic cells (BMDC) with particles harboring Anx as well as the model antigen ovalbumin (OVA) attenuated the response of OVA-specific OT-II T cells. The co-culture of Anx-particletreated DC and T cells resulted in an anergy-like phenotype characterized by reduced proliferation and cytokine secretion. Here we demonstrate that the anti-inflammatory effects of Anx which are mediated through DC can be used as a tool to generate a particle-based antigen delivery system that promotes antigen-specific immunosuppression. Such Anx-particles may be a new therapeutic approach for the treatment of autoimmune disease.

Curvature induction lipid membranes by ANXA5 and a Membrane Potential: Insights from Molecular Simulations

Himanshu Khandelia

Annexins are a family of Ca2+-dependent, membrane-binding proteins active in membrane repair mechanisms throughout Eukarya. We investigate the binding of rat Anxa5 to a lipid membrane composed of 20% anionic lipids (POPS and POPC, 1:4) in silico using molecular dynamics. Anxa5 showed calcium-dependent binding to negatively charged lipids and contained four annexin repeats. Binding of annexin binding resulted in a thinning of the membrane and a negative membrane curvature of 0.2 nm-1. Glutamate and aspartate residues in the regions closest to the membrane are active in calcium-binding, and that lysine and methionine residues in the same regions interact with the membrane to favour binding in addition to the calcium-membrane interactions. The results are in agreement with biophysical measurements of membrane rolling induced by Anxa5, in the direction of the annexin-bound surface.
We will also report on how a transmembrane electrostatic potential, can also induce curvature in a simple membrane as a result of electromechanical coupling in the membrane.

ANXA5, Molecular dynamics, membrane curvature, membrane repair, electromechanical coupling

Ligand bias at the formyl peptide receptor: Impact on inflammation balance

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Excessive leukocyte activation and transendothelial migration are often linked to failed inflammatory resolution and the establishment of chronic disease; hence, the detailed understanding of key players in these carefully orchestrated mechanisms may lead to novel therapeutic targets for the treatment of leukocyte-driven inflammatory disorders. Leukocytes sense and respond to inflammatory stimuli including pathogen-associated molecular pattern molecules (PAMPs) derived from microorganisms, and endogenous, cell-derived damage-associated molecular pattern molecules (DAMPs). A specialized small family of pattern recognition receptors (PRRs), the G protein-coupled Formyl Peptide Receptors (FPRs), recognize bacterial (PAMPs) and mitochondrial (DAMPs) N-formylated peptides, but also a range of chemically unrelated, non-formylated endogenous compounds, including Annexin A1 (AnxA1). To explore the use of the FPR signal axis as a means to induce a tailored and beneficial response, we started to systematically analyze and compare the agonist-specific signaling fingerprints of these receptors. We already uncovered "biased agonism" at the FPRs, with agonist clusters defined by the DAMP or PAMP origin of the formylated peptides.

Lack of endogenous annexin A1 exacerbates NLRP3 inflammasome response in macrophages

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This study evalluated the role of endogenous Annexin A1 in the regulation of the NLRP3 inflammasome. B57bl/6 wild-type (WT) and knockouts for ANXA1 (ANXA1-/-) received intraperitoneal injection of 1.5% starch solution for macrophages recruitment. NLRP3 was activated by priming cells with lipopolysaccharide (500 ng/mL) for 3 hours, followed by nigericin (10 μM for 1 hour) or ATP (5 mM for 30 minutes). After the treatments, the cells and supernatant were collected for ELISA, western blotting, cell viability by MTT, ultrastructural immunocytochemistry and, lipidomic analysis. As expected, nigericin and ATP administration decreased macrophage viability, more pronounced in the ANXA1-/- cells, compared with control and only LPS-treated cells ($p < 0.001$). Additionally, treatment with nigericin and ATP produced a marked release of IL-1β in ANXA1-/- macrophages compared to the other groups. Ultrastructural analysis demonstrated a high expression of NLRP3 in the nigericin-activated ANXA1-/- macrophages. WT cells showed points of co-localization of ANXA1 and NLRP3. Lipidomic analysis of macrophages evidenced a completed different lipid profile between WT and ANXA1-/- supernatant cells. In WT, nigericin administration induced a pronounced release of eicosanoids and prostaglandins, while ANXA1-/- showed precursors of prostaglandin and some ceramides. Altogether, our results suggested that endogenous ANXA1 regulates the NLRP3-derived IL-1β in macrophages.

Keywords: ATP, immunoelectron microscopy, lipidomic analysis, nigericin, pyroptosis.

Support: FAPESP (2017/26872-5); CAPES (Finance Code 001); CAPES/PRINT (88881.310737/2018-01).

Poster 10

Annexin A1 and formyl peptide receptors signaling are crucial to the efficacy of infliximab therapy during intestinal inflammation

Marina De Paula Silva

Infliximab (IFX) is an anti-TNF-α therapy to inflammatory bowel diseases. IFX induces annexin A1 (AnxA1) expression in patients, however, AnxA1 and its formyl-peptide receptors (FPRs) effects over IFX efficacy are unknown. We assessed the roles of FPRs and AnxA1 over IFX mechanisms on intestinal inflammation. Male C57BL6 wild-type (WT) and AnxA1-deficient (AnxA1-/-) mice received 2% dextran sulphate sodium (DSS) in drinking water at Day 0 and were treated with Boc-2 (FPRs pan-blocker; 10 ug/mL) or IFX+Boc-2 intraperitoneally. DSS was withdrawn at Day 6 and mice were euthanized at Day 10. In vitro, Caco-2 epithelial monolayers were stimulated with TNF-αand treated with IFX alone, with FPR1 (Boc-2) or FPR2 (WRW4) antagonists. In WT mice, IFX prevented clinical parameters of disease; while concomitant treatment with FPRs antagonists intensified them. AnxA1 expression increased on WT/DSS and WT/DSS+Boc-2 damaged epithelium. IFX increased AnxA1 colonic secretion, with higher concentrations correlated with disease symptomatology. AnxA1-/- mice presented more severe disease, with a 50% mortality after IFX. IFX protected tight junctions and prevented TNF-αreceptor 2 increase in Caco-2 cells; FPR1 and FPR2 blockades reverted IFX effects. In conclusion, FPRs participate on IFX protection over epithelium, and AnxA1 is a crucial FPR ligand to experimental colitis resolution.

Keywords: inflammatory bowel disease, dextran sulphate sodium, Caco-2, infliximab, biomarker

Poster 11

Membrane rolling induced by AB5 toxins

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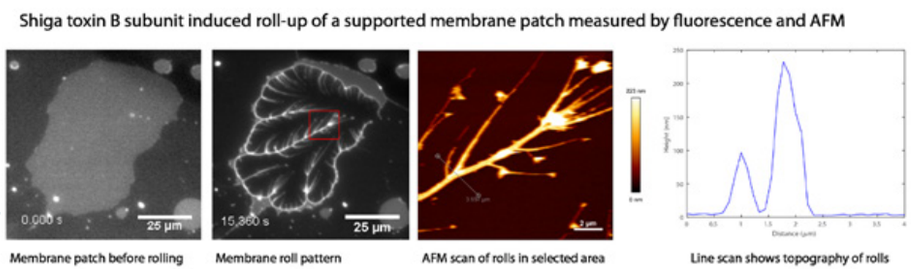
Many types of membrane interacting proteins induce spontaneous curvature upon membrane binding. Shiga and Cholera toxins both belong to the AB5 family of toxins and both consist of a toxic (active) A subunit and a membrane-binding pentameric B subunit. It is known that Shiga and Cholera toxins induce tubular membrane invaginations in GUVs due to curvature effects. The toxins are also known to induce curvature from MD simulations. This is modeling endocytosis of the toxin through the plasma membrane of cells.

We have recently studied Annexins which is another class of curvature-inducing proteins. Annexins have important functions in plasma membrane repair where curvature near hole edges is a key event in the repair process.

As a model system to characterize curvature-inducing proteins, we study the morphology induced in planar membrane patches. It was previously shown that Annexins induce characteristic morphologies in membrane patches including membrane rolling.

In this study we show that the B subunits of Shiga and of Cholera toxins (STxB, CTxB) both induce roll-up of cell-sized membrane patches. Rolling starts from the free membrane edges of the patch and is completed within a few seconds. We quantify the rolling speed and morphology of rolls induced by the toxins. Our results indicate that membrane rolling may be a general effect displayed by many proteins that induce negative curvature.

Keywords: Curvature-induction, Shiga, Cholera, Annexins



Annexin A4 inhibits the activation of the intrinsic blood coagulation pathway

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Background: Blood coagulation process plays an essential role in stopping the bleeding. However, it can also cause severe thrombosis. The intrinsic coagulation pathway is initiated by the contact activation of coagulation factor XII (FXII) triggered by negatively charged surfaces (e.g. sulfatide). Recently, it has been clarified that the intrinsic pathway is involved in thrombus formation but not in physiological hemostasis. Annexin (ANX) A4 is considered as a potent anticoagulant, but its role in the inhibition of intrinsic pathway activation is unclear and was investigated in this study.

Results and findings: Our data showed that ANXA4 inhibits sulfatide-triggered coagulation in plasma suggesting that it regulates the intrinsic pathway. Enzymatic activity measurements indicated that ANXA4 inhibits FXII and FXI activation. While sulfatide binding to ANXA4 was suspected to trigger this inhibition, amino acid substitution of sulfatide binding residues did not abolish the anticoagulant activity. ANXA4 consists of four repeat domains, and among them, repeat 3 and repeat 4, were tested for their involvement in the anticoagulant activity. The anticoagulant test performed by using the recombinants lacking repeat 3 (ΔR3) or 4 (ΔR4) revealed that only ΔR4 exhibits reduced anticoagulant activity and reduced inhibition of FXII and FXI. These results demonstrate that repeat 4 of ANXA4 contributes to the regulation of the intrinsic coagulation pathway by inhibiting FXII and FXI.

Keywords: ANXA4, anticoagulation, intrinsic pathway, sulfatide

Characterisation of Annexin A11 expression using an in-vitro stable TRSY cell line

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King's College London

Background: We recently identified novel Annexin A11 mutations in 751 familial ALS cases driven by a common D40G mutation (1).
Objective: To study Annexin A11 expression we generated a tetracycline-repressor SH-SY5Y (TRSY) doxycycline inducible stable cell line expressing haemagglutinin (HA-) tagged WT Annexin A11 and D40G. Cell lines were differentiated
into neuronal like cells (2) and grown to days in vitro (DIV) 6 and DIV 10 to assess expression levels of Annexin A11.
Methods: ANXA11-TRSY mixed population cells were dox activated for 24 hours and fixed in 4 % paraformaldehyde (PFA) for downstream immunocytochemistry (ICC), confocal microscopy and western blotting. TRSY cells were differentiated using EC23 supplemented media for 3 days changed to Neurobasal media for culture maintenance.
Results:Dox activated stable WT and D40G cell lines demonstrated Annexin A11 expression. Of note, differentiated cell lines had increased expression of Annexin A11 within neuronal-like cell bodies and axons for both WT and D40G
between DIV 6 and DIV 10.
Discussion and Conclusions: Differentiated TRSY stable lines displayed a marked expressional increase of Annexin A11 (DIV6 to DIV10) suggesting that Annexin A11 expression may be a temporal as well as cell-type dependent phenomenon.

References
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Keywords: ALS, Annexin A11

The Annexin A1/FPR2 signaling axis expands alveolar macrophages, limits viral replication, and attenuates pathogenesis in the murine influenza A virus infection model

Sebastian Schloer

Pattern recognition receptors (PRRs) are key elements in the innate immune response. Formyl Peptide Receptor 2 (FPR2) is a PRR that in addition to proinflammatory, pathogen-derived compounds, also recognizes the anti-inflammatory endogenous ligand Annexin A1 (AnxA1). Because the contribution of this signaling axis in viral infections is undefined, we investigated AnxA1-mediated FPR2 activation on Influenza A virus (IAV) infection in the murine model. AnxA1-treated mice displayed significantly attenuated pathology upon a subsequent IAV infection with significantly improved survival, impaired viral replication in the respiratory tract, and less severe lung damage. The AnxA1-mediated protection against IAV infection was not caused by priming of the type I interferon (IFN) response but was associated with an increase in the number of alveolar macrophages (AMs), and enhanced pulmonary expression of the AM-regulating cytokine Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). Both AnxA1-mediated increase in AM levels and GM-CSF production were abrogated when mFPR2 signaling was antagonized but remained upregulated in mice genetically deleted for mFPR1, an mFPR2 isoform also serving as AnxA1 receptor. Our results indicate a novel protective function of the AnxA1-FPR2 signaling axis in IAV pathology via GM-CSF-associated maintenance of AMs, expanding knowledge on the potential use of pro-resolving mediators in host-defense against pathogens.

Keywords: Pattern recognition receptors, innate immune system, mucosal immunity

Annexin A8 knockout impairs Influenza A Virus replication and propagation but also intervene with the innate immunity

Sebastian Schloer

The release of viral genome into the host cytoplasm and its translocation to the nucleus is strongly depended on the fusion of Influenza A virus (IAV) envelope with host endosomal membranes. Recent publications have already delineated the importance of cellular cholesterol levels on the infectivity of IAV. Here, we surveyed the role of the Ca2+-dependent membrane-binding protein Annexin A8 (AnxA8) for the functionality of cellular membranes and their lipid composition in the context of IAV infection. The knockout of AnxA8 shifted peripheral cholesterol levels towards the endolysosomal system (LE/L) that goes along with reduced IAV infectivity. Our findings identify AnxA8 as a regulator of LE/L cholesterol balance, restricting incoming IAV particles by influencing the fusion of LE/L and viral membrane. As cholesterol is also a critical and limiting factor for IAV virus propagation and budding, we further performed electron microscopy to study the budding and release of newly formed IAV particles in AnxA8 knockout cells. Our results indicate a protective function of LE/L cholesterol accumulation in IAV-infected cells as a potent antiviral target. Interestingly, AnxA8KO mice are highly susceptible for IAV infection which correlates with higher mortality and increased viral burden in the respiratory tract. A deeper view into the immune response upon IAV infection in primary murine lungs cells revealed that increased viral replication in AnxA8KO cells is linked to lower cytokine levels.

Keywords: annexin A8, atomic force microscopy, cholesterol, endolysosome, electron microscopy, influenza A infection,

Role of protein annexin a1 in human placental response to maternal zika virus infection

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Recent studies have shown that the Annexin A1 (AnxA1) protein is involved in the resolution of inflammation through multiple mechanisms and associated with placental infections. In light of the above reports, the aim of our investigation was to evaluate inflammatory process and the involvement of ANXA1 in placentas of ZIKV-infected mothers. Placental fragments were analyzed for the presence of ZIKV and classified in three study groups: Neg/Neg (mother/placenta negative for the virus), Pos/Neg (infected mother, but no virus detected in placenta) and Pos/Pos (mother/placenta infected with ZIKV). The presence of ZIKV in the Pos/Pos group shows lower gestational age at delivery, their newborns had the lowest weight and placentas with structural alterations, including detachment and disorganization of syncytiotrophoblast. Maternal infection by ZIKV (regardless of placenta infection) triggered a placental inflammatory response, observed by recruitment of inflammatory cells and increased levels of cytokines IL-1β, IL-8, IL-10 and TNF-α in placenta. In the syncytiotrophoblast, ANXA1 was expressed in nuclei and cytoplasm, and its expression was decreased in mother/placenta infected specimens. These results envisage that maternal infection with ZIKV is able to develop a placental inflammatory response, possibly related to the negative ANXA1 modulation. Our study sheds additional light on the outcomes of ZIKV infection in trophoblast and in the involvement of ANXA1 in placental biology.

Keywords: Human Placenta; Syncytiotrophoblast; Inflammation; Neutrophils; Annexin A1

Characterization of a Zebrafish misexpression and CRISPR knockout model of the ALS associated gene Annexin A11

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Amyotrophic lateral sclerosis (ALS) is a late onset, neurodegenerative disorder, with an average survival of 2-5 years post diagnosis. The majority of cases are sporadic, however up to 10% are inherited and attributable to mutations in approximately 25 causative genes. We have recently identified novel mutations in Annexin A11 (encoded by ANXA11) in a large exome sequencing study of familial ALS patients (n=751), in which the most common mutation, D40G, was found 6 times in European patients and segregated with disease in two large UK kindreds. Post mortem tissue from a patient harbouring the D40G mutation after ANXA11 immunostaining was found to harbour prolific, large insoluble aggregates in the spinal cord and motor cortex (Smith et.al, 2017). To investigate disease mechanism we have created a neuron specific in-vivo over-expression model (UAS ANXA11-GFP; MNX1:GAL4) to investigate Wild Type Human ANXA11 and the predominant ANXA11 mutation p.D40G in zebrafish motor neurons and a loss of function CRISPR knockout model of the Zebrafish orthologue Annexin A11A. Our results were striking in that larval D40G mutants at 48hpf presented with a curved tail, haemorrhages, blood accumulation and severe pericardial oedema with no differences observed in WT Annexin A11. Furthermore, D40G mutants showed significantly shorter motor axons and a decrease in the number of axonal branches as well as neuromuscular junction disruption. The knockout line was produced by standard CRISPR/Cas9 methods targeting a region of the N-terminus of Annexin A11A. F2 progeny were crossed to produce F3 larvae that were either WT, heterozygous or homozygous for the knockout mutation. Phenotypic characterization of this stable Annexin A11A CRISPR line revealed that the mutation has a low penetrance at 48hrs with 25% of homozygous larvae displaying multiple abnormal phenotypes: curvy tail, curvature in the spinal cord, aberrant posterior structure formation and increased pigmentation, with dispersed melanin on their heads and backs. Also, homozygous embryos displayed also motor impairment and neuromuscular junction defects. In conclusion, the D40G over-expression model, which is toxic to neuronal development, putative function and morphology, does recapitulate some aspects of ALS. Furthermore, the loss of function study has demonstrated that Annexin A11A is important for Zebrafish neuronal function. Lastly, these results validate the potential usefulness of zebrafish as a model for investigating ALS pathogenesis in the case of Annexin A11.

Keywords: amyotrophic lateral sclerosis, Annexin A11, in-vivo, zebrafish, CRISPR knockout

List of Speakers

Poster Presenters