Program and Abstract Book

Transglutaminases in Medicine
Debrecen University Symposium 2017

Cell Death & Differentiation

DOTE Apoptosis Research Foundation.

BIO-SCIENCE
A magyar tudomány szolgálatában.

zedia covalab
R&D in Biotechnology

U20 év

DEBRECEN UNIVERSITY SYMPOSIUM 2017
Map - walking directions from hotels.

Cover illustration: Overlay of the structural models of the 'open' conformations of TG2 (blue) and FXIII-A (orange).
PDB: 2Q3Z and 4KTY.
Design by Máté Demény

DEBRECEN UNIVERSITY SYMPOSIUM

TRANSGLUTAMINASES IN MEDICINE

2017 August 3-5

Venue:

Hotel Divinus (August 3)

University of Debrecen Life Science Building (August 4-5)
Scientific Advisory Board:

*László Fésüs (chair), László Muszbek, Ilma Korponay-Szabó, Zsuzsa Szondy*

Local organizers

*Nóra Elek, Róbert Király, Universitas GmbH (Timea Lilik and Erika Benke)*

The symposium chair and the organizers are grateful to the university choosing “Transglutaminases in Medicine” as the topic for the 2017 Debrecen University Symposium.

Generous supports from sponsors - DOTE Apoptosis Research Foundation, the journal Cell Death and Differentiation, BioScience, Covalab, UD-GenoMed GmbH, Zedira - are highly appreciated.

**Practical information:**

Registration will open on August 3, Thursday at 16:00 in Hotel Divinus

Buses will arrive from the airport to Hotel Divinus from where other hotels are in walking distance or can be reached by tram (a few stops)

On August 4 (Friday) participants will be escorted from their hotel by young colleagues of the Department of Biochemistry and Molecular Biology of the University to the Life Science Building lecture hall which is approximately 15-20 minutes by walk. Meet them in the hotel lobbies at 8:30 am.

Evening program on August 4: Buses will leave at 19:30 from hotel Divinus

Farewell party on August 5 will take place in the Water Tower, located only a few minutes from Hotel Divinus.
Dear Friends and Colleagues in Transglutaminase Research,

It is a great pleasure to welcome you at the Debrecen University Symposium “Transglutaminases in Medicine” dedicated to four decades of transglutaminase research in Debrecen and to mark overall progress in this exciting area of biomedical sciences.

The interest of several research groups in various departments and clinics at our university has turned to transglutaminases during the years attracting generations of talented young researchers to join the field.

Worldwide transglutaminase research has reached a new phase of development contributing to better understanding pathomechanism of a broad range of diseases as well as leading to novel diagnostic possibilities and innovative therapeutic strategies.

This is not the first transglutaminase meeting in Debrecen. The “Fourth Transglutaminase Conference on Transglutaminases and Protein Cross-linking Reactions” with 115 participants was held here in August 1994. We were fortunate to have pioneers of the field among the speakers including Laszlo Lorand, Ariel Loewy, Bob Graham, Peter Davies and Peter Steinert. The first transglutaminase structure, of the FXIIIa zymogen, was introduced by Vivien Yee during the conference. The meeting covered broad areas including novel transglutaminases, inheritable transglutaminase diseases, transcriptional regulation of TGase genes, apoptosis, involvement of TGase family members in disease pathogenesis and significance of protein cross-linking in tissue homeostasis. I strongly hope that the rich program of the current symposium offers similarly memorable scientific exposures leading to highly valuable discussions.

The social programs coupled to the Symposium, the university campus and cultural offerings of the city provide attractions to ensure you enjoy the days spent with us as much as possible.

László Fésüs Symposium chair
## PREVIOUS TRANSGLUTAMINASE CONFERENCES

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<thead>
<tr>
<th>Host/Chair</th>
<th>Keynote address/Opening lecture</th>
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<tr>
<td>MIAMI 1988</td>
<td>Peter Davies Russel Doolittle</td>
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<td>CANNES 1990</td>
<td>Rainer Schmidt, Uwe Reichert Pierre Chambon</td>
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<td>ARDMORE 1992</td>
<td>Paul Birkhichler Howard Green</td>
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<tr>
<td>DEBRECEN 1994</td>
<td>László Fésüs Laszlo Lorand, László Patthy</td>
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<tr>
<td>CHEJU 1996</td>
<td>Ron Chung Ron Chung (a tribute to Jack Folk)</td>
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<td>LYON 2000</td>
<td>Said El Alaoui, Gerard Quash Peter Steinert</td>
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<td>FERRARA 2002</td>
<td>Carlo Bergamini Laszlo Lorand</td>
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<td>LÜBECK 2005</td>
<td>Rolf Hilgenfeld Chaitan Khosla, Ursula Quitterer</td>
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<td>MARRAKECH 2007</td>
<td>Said El Alaoui László Fésüs</td>
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<td>GORDON CONFERENCES</td>
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<tr>
<td>2010 Richard Eckert</td>
<td>László Fésüs, Martin Griffin</td>
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<td>2012 Kapil Mehta</td>
<td>Peter Davies</td>
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<td>2014 Mauro Piacentini</td>
<td>László Fésüs, Peter Vandenabeele</td>
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<td>2016 Tim Johnson</td>
<td>Mauro Piacentini, Kapil Mehta, Benjamin Drukarch</td>
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SCIENTIFIC PROGRAM
August 3 Thursday  
Venue: Hotel Divinus

OPENING  
17:45-18:00  
József Tőzsér vice rector, University of Debrecen  
László Fésüs

WELCOME TO DEBRECEN TRANSGLUTAMINASE RESEARCH AT FORTY YEARS  
18:00-19:30  
Chair: Mauro Piacentini

László Fésüs  
University of Debrecen  
MEDICINE ORIENTED TRANSGLUTAMINASE RESEARCH – WHERE HAVE WE BEEN AND WHERE ARE WE GOING?  
18:00-18:20  
Discussion  
18:20-18:30

László Muszbek  
University of Debrecen  
COAGULATION FACTOR XIII: A PROTRANSGLUTAMINASE WITH FUNCTIONS OUTSIDE THE COAGULATION CASCADE  
18:30-18:50  
Discussion  
18:50-19:00

László Nagy  
University of Debrecen and Sanford Burnham Prebys Medical Discovery Institute at Lake Nona  
THE GENE REGULATION OF TISSUE TRANSGLUTAMINASE AS A MODEL OF THE INTERACTION OF SIGNAL AND LINEAGE SPECIFIC TRANSCRIPTIONAL CONTROL  
19:00-19:20  
Discussion  
19:20-19:30

WELCOME RECEPTION at Hotel Divinus  
19:30
**August 4 Friday**  
Venue: Lecture Hall, Life Science Building University of Debrecen

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<th>Title</th>
<th>Speaker</th>
<th>University</th>
<th>Time</th>
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<td><strong>9:00-11:00</strong></td>
<td>HAEMOSTASIS – FACTOR XIII</td>
<td>Chair: László Muszbek</td>
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<td>Robert Ariens</td>
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<td>University of Leeds</td>
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<td>Arijit Biswas</td>
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<td>University Clinic Bonn</td>
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<td>Cedric Duval</td>
<td>10:00-10:10</td>
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<td>University of Leeds</td>
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<td>Éva Katona</td>
<td>10:15-10:25</td>
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<td>University of Debrecen</td>
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<td>Anna Bychkova</td>
<td>10:30-10:40</td>
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<td>N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow</td>
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<table>
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<tr>
<th>Discussion</th>
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<td>9:20-9:30</td>
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<td>9:50-10:00</td>
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<td>10:40-10:45</td>
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<tr>
<td>10:45-10:55</td>
<td><strong>BLOOD COAGULATION FACTOR XIII IN TEARS</strong>&lt;br&gt;And Its Possible Role in Corneal Wound Healing&lt;br&gt;Discussion</td>
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<td>10:55-11:00</td>
<td><strong>COFFEE BREAK</strong></td>
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<td>11:00-11:30</td>
<td><strong>TISSUE REMODELLING AND FIBROSIS</strong>&lt;br&gt;Chair: Elisabetta Verderio</td>
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<td>11:30-11:50</td>
<td><strong>Siiri E Iismaa</strong>&lt;br&gt;<strong>Victor Chang Cardiac Research Institute</strong>&lt;br&gt;TG2 AND TISSUE REMODELLING&lt;br&gt;Discussion</td>
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<td>11:50-12:00</td>
<td><strong>Martin Griffin</strong>&lt;br&gt;<strong>Aston University</strong>&lt;br&gt;The Functional Relationship Between TG2 And TGFβ1 And Its Importance As A Therapeutic Target In Human Disease&lt;br&gt;Discussion</td>
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<td>12:00-12:20</td>
<td><strong>Timothy Johnson</strong>&lt;br&gt;<strong>University of Sheffield</strong>&lt;br&gt;TG2 Inhibitory Therapy In The Treatment Of Fibrotic Remodelling: A Translation Journey&lt;br&gt;Discussion</td>
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<td>12:20-12:30</td>
<td><strong>Discussion</strong></td>
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<td>12:30-12:50</td>
<td><strong>LUNCH AND POSTER VIEWING, DISCUSSION</strong></td>
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All posters will be shown throughout the symposium. On the first day, presenting authors of P1-P13 are expected to be present at their poster between 13:30-14:15. There will brief (3 minutes) oral presentation of posters P2, P6, P8, P10 and P12 by authors at poster boards.
SKIN DISEASES

Chair: Richard Eckert

Eleonora Candi
University of Rome “Tor Vergata”
TRANSGLUTAMINASE 3 PROTECTS AGAINST PHOTODAMAGE
Discussion

Hitomi Kiyotaka
Nagoya University
ANALYSES OF TRANSGLUTAMINASES ACTIVITIES AND THEIR SUBSTRATES USING SPECIFIC SUBSTRATE PEPTIDES
Discussion

Dániel Törőcsik
University of Debrecen
TRANSGLUTAMINASE 2 IS A POTENTIAL REGULATOR OF SEBOCYTE MATURATION
Discussion

Samuel Hjorth-Jensen
Queensland University of Technology
THE DYNAMIC NATURE OF TRANSGLUTAMINASES
Discussion

COFFEE BREAK

CANCER

Chair: Carlo Bergamini

Richard Eckert
University of Maryland
TG2 DRIVES NOVEL MECHANISMS OF CANCER STEM CELL SURVIVAL AND TUMOR FORMATION
Discussion
Kapil Mehta
The University of Texas MD Anderson Cancer Center and Lifecare Innovations, Gurugaon

TARGETING TRANSGLUTAMINASE II (TG2) FOR CANCER TREATMENT – HYPES AND HOPES
Discussion

János Kappelmayer
University of Debrecen

INTRACELLULAR FACTOR XIII IS A PROGNOSTIC MARKER IN PEDIATRIC LYMPHOBLASTIC LEUKEMIA
Discussion

Xian-Yang Qin
RIKEN Center for Life Science Technologies, Wako

ROLE OF TRANSGLUTAMINASE 2 IN SELECTIVE DELETION OF MYCN+CD133+ LIVER CANCER STEM CELLS BY ACYCLIC RETINOID
Discussion

Robert Wodtke
Helmholtz-Zentrum Dresden-Rossendorf

RADIOSYNTHESIS AND IN VITRO CHARACTERISATION OF A POTENT 18F-FLUORINATED Nε-ACRYLOYLLYSINE AS ACTIVITY-BASED PROBE FOR TRANSGLUTAMINASE 2
Discussion

COUNTRY SIDE FOLKLORE PROGRAM WITH DINNER AT FRATER RANCH
August 5 Saturday  
Venue: Lecture Hall, Life Science Building University of Debrecen

**NEURODEGENERATION**

Chair: Martin Griffin

**Gail VW Johnson**
*University of Rochester*

DELETION OF ASTROCYTIC TRANSGLUTAMINASE 2 IMPROVES OUTCOMES SUBSEQUENT TO INJURY

Discussion

**Philippe Djian**
*Université Paris Descartes*

TRANSGLUTAMINASE AND NEUROLOGICAL DISEASE: A STUDY BY FUNCTIONAL PROTEOMICS

Discussion

**Anne-Marie Van Dam**
*VU University Medical Center, Amsterdam*

MONOCYTE-DERIVED TISSUE TRANSGLUTAMINASE IN MULTIPLE SCLEROSIS PATIENTS: REFLECTING AN ANTI-INFLAMMATORY STATUS OF THE CELLS?

Discussion

**Elisabetta Verderio**
*Nottingham Trent University*

THE TRANSGLUTAMINASE-2 “TRANSAMIDOME” IN PRIMARY HIPPOCAMPAL NEURONS EXPOSED TO AMYLOID-β PEPTIDE (Aβ)

Discussion

**COFFEE BREAK**
GASTROINTESTINAL DISEASES – CELLIAC DISEASE AND HEPATITIS
11:30-13:00
Chair: Siiri Iismaa

Ludvig Sollid
University of Oslo
AUTOIMMUNITY TO TRANSGLUTAMINASE 2 IN CELLIAC DISEASE
Discussion
11:30-11:50

Ilma Korponay-Szabó
University of Debrecen and Heim Pál Children’s Hospital, Budapest
ROLE OF TRANSGLUTAMINASE 2 EPITOPES IN THE SELECTIVE DIAGNOSIS OF CELLIAC DISEASE
Discussion
12:00-12:20

Soichi Kojima
RIKEN Center for Life Science Technologies, Wako
CONTROL OF NUCLEAR TG2 IN HEPATIC DISEASE
Discussion
12:30-12:50

LUNCH AND POSTER VIEWING, DISCUSSION
13:00-14:30
All posters will be shown throughout the symposium. On the second day, presenting authors of P14-P26 are expected to be present at their poster between 13:30-14:15. There will brief (3 minutes) oral presentation of selected posters by authors at poster boards.

STRESS, OBESITY AND INFLAMMATION
14:30-16:00
Chair: Ludvig Sollid

Mauro Piacentini
University of Rome "Tor Vergata"
TYPE 2 TRANSGLUTAMINASE: A KEY REGULATOR OF PROTEOSTASIS UNDER CELLULAR STRESSFUL CONDITIONS
Discussion
14:30-14:50
Zsuzsa Szondy  
*University of Debrecen*

**LOSS OF TRANSGLUTAMINASE 2 SENSITIZES FOR THE DEVELOPMENT OF INFLAMMATION AND INSULIN SENSITIVITY IN MICE KEPT ON HIGH FAT DIET**  
Discussion  
15:00-15:20

András Mádi  
*University of Debrecen*

**BROWNING DEFICIENCY AND LOW MOBILIZATION OF TRIACYLGlycerols in GONADAL WHITE ADIPOSE TISSUE LEADS TO DECREASED COLD-TOLERANCE OF TRANSGLUTAMINASE 2 KNOCK-OUT MICE**  
Discussion  
15:30-15:40

Károly Jambrovics  
*University of Debrecen*

**PATHOBIOLOGICAL FUNCTION OF TRANSGLUTAMINASE 2 IN DIFFERENTIATING NB4 ACUTE PROMIELOCYTIC LEUKAEMIA CELLS**  
Discussion  
15:45-15:55

COFFEE BREAK  
16:00-16:30

**PERSPECTIVES**  
Chair: Timothy Johnson

Jeffrey Keillor  
*University of Ottawa*

**TARGETED COVALENT INHIBITION OF TISSUE TRANSGLUTAMINASE IN CANCER STEM CELLS**  
Discussion  
16:30-16:50

Said El Alaoui  
*Covalab, Lyon*

**COVISOLINK™: NOVEL SITE-SPECIFIC ADCs GENERATION USING mTG CROSSLINKING**  
Discussion  
17:00-17:20
Daniel Aeschlimann  
Cardiff University

UNCONVENTIONAL PROTEIN SECRETION IN INNATE IMMUNITY, A NEW LINK BETWEEN TRANSLGLUTAMINASE 2 AND DISEASE PROCESSES

Discussion

CONFERENCE CLOSING

SYMPOSIUM PHOTO in front of the main building of the University

FAREWELL PATY WITH LIVE MUSIC AND DANCE AT WATER TOWER.

POSTERS

TISSUE TRANSGLUTAMINASE CORRELATES WITH DISEASE PROGRESSION AND EPITHELIAL-MESENCHYMAL TRANSITION IN COLORECTAL CANCER CELLS  
Oluseyi Ayinde  
Aston University

RNA ANALYSIS INDICATES TIGHT CORRELATION BETWEEN THE EXPRESSION OF A LONG NON CODING RNA AND THE TG2 mRNA LEVELS  
Nicoletta Bianchi  
Universita` di Ferrara Consorzio Futuro in Ricerca

SMART COLLAGEN MATRICES INCORPORATING BIOACTIVE GLASS FOR USE IN BONE HEALING AND REGENERATION  
Haris Choudhery  
Aston University
THE INTERACTION BETWEEN TG2 AND SYND ECAN-4 IS CRITICAL FOR TG2 AND FIBRONECTIN MATRIX DEPOSITION IN LUNG FIBROSIS
Shaun Alexander Fell
Aston University

A FLUORESCENCE ANISOTROPY-BASED ASSAY TO CHARACTERIZE THE GTP-BINDING SITE OF TISSUE TRANSGLUTAMINASE
Christoph Hauser
University of Cologne

DEVELOPMENT AND CHARACTERIZATION OF HUMAN MELANOMA CELL LINES AND XENOGRAFT MODELS EXHIBITING DIFFERENT LEVELS OF TRANSGLUTAMINASE 2
Sandra Hauser
Helmholtz-Zentrum Dresden-Rossendorf

A VALIDATED ALL ATOM MODEL FOR THE COAGULATION FACTOR XIII A2B2 HETERTETRAMERIC COMPLEX
Sneha Singh
University Clinic Bonn

INCREASED ACTIVITY OF HUMAN TRANSGLUTAMINASE 2 IN THE ERYTHROCYTES OF PATIENTS WITH SICLE CELL DISEASE
Sándor Sipka
University of Debrecen

COVISOLINK™: NEW BACTERIAL TRANSGLUTAMINASE Q-TAG SUBSTRATE FOR THE DEVELOPMENT OF SITE SPECIFIQUE ANTIBODY DRUG CONJUGATES
Éva Sivadó
Covalab Lyon
TRANSGLUTAMINASE ACTIVITY REGULATES DIFFERENTIATION, MIGRATION AND FUSION OF OSTEOCLASTS VIA AFFECTING ACTIN DYNAMICS AND RHOA ACTIVITY
Hoifang Sun
McGill University

CHARACTERISATION OF THE ISOPEPTIDASE ACTIVITY OF HUMAN BLOOD COAGULATION FACTOR XIII-A
Zsuzsa Szabó
University of Debrecen

DRUG-RESISTANCE OF TISSUE TRANSGLUTAMINASE TRANSAMINASE AND GTP-BINDING FUNCTIONS IN RENAL CELL CARCINOMA
Burge Ulukan
Yeditepe University

THE FUNCTIONAL RELATIONSHIP BETWEEN TG2 AND TGFβ1 AND ITS IMPORTANCE AS A THERAPEUTIC TARGET IN HUMAN DISEASE
Zhuo Wang
Aston University

TRANSGLUTAMINASE TYPE 2 INTERACTS WITH GRP75 AND INFLUENCES THE Ca2+ HOMEOSTASIS AT THE ER/MITOCHONDRIA (MAM) CONTACT SITES
Manuela D'Eletto
University of Rome "Tor Vergata"

ATTACHMENT OF SMALL PROTEIN MODIFIERS BY TRANSGLUTAMINASES
Máté Demény
University of Debrecen

UNEXPECTED ROLE OF N-TERMINAL β-SANDWICH AND CORE DOMAINS’ INTERPLAY IN TG2 FUNCTIONS
Rita Elek
University of Debrecen
THE ROLE OF P2X7R IN TRANSGLUTAMINASE 2 EXPORT AND ACTIVATION: IMPLICATIONS FOR DISEASES RELATED TO IMMUNITY
Rhiannon Griffiths
Cardiff University

THE DYNAMIC NATURE OF TRANSGLUTAMINASES
Samuel Hjorth-Jensen
Queensland University of Technology

LIPOMA-PREFERRED PARTNER PROTEIN (LPP), A GENETIC MARKER FOR COELIAC DISEASE PREDISPOSITION IS INTERACTING WITH TRANSGLUTAMINASE 2 (TG2)
Boglárka Kerekesné Tóth
University of Debrecen

STUDYING BIOLOGICAL SIGNIFICANCE OF TRANSGLUTAMINASE 2 BY COMPARING FUNCTIONS OF HUMAN AND MOUSE RECOMBINANT ENZYMES
Róbert Király
University of Debrecen

ADRENERGIC AGONIST FAILS TO INDUCE BROWNING IN EPIDIDYMAL FAT OF TISSUE TRANSGLUTAMINASE KNOCK-OUT MICE
Kinga Lénárt
University of Debrecen

ANALYSIS OF THE PROTEIN CROSSLINK-PROFILE CHANGES AND THE NEUTROPHIL EXTRACELLULAR TRAP PATTERNS ELICITED BY DIFFERENT STIMULI
Bernadett Márkus
University of Debrecen

STRUCTURE-ACTIVITY RELATIONSHIPS OF POTENT, TARGETED COVALENT INHIBITORS THAT ABOLISH BOTH THE TRANSAMIDATION AND GTP BINDING ACTIVITIES OF HUMAN TISSUE TRANSGLUTAMINASE
Nicole McNeil
University of Ottawa
UPDATE TO TRANSDAB – A COLLECTION OF TRANSGLUTAMINASE SUBSTRATES AND INTERACTION PARTNERS
Timea Székely
*University of Debrecen*

INVOLVEMENT OF TG2 IN CALCIUM HOMEOSTASIS IN RAT PRIMARY HIPPOCAMPAL NEURONS
Elisa Tonoli
*Nottingham Trent University*

UNCONVENTIONAL EXPORT OF TRANSGLUTAMINASE 2: ROLE OF CO-FACTORS REGULATING ENZYME ACTIVITY AND CONFORMATIONAL STATE
Shannon Turberville
*Cardiff University*
ABSTRACTS – ORAL PRESENTATIONS
Similarly to many other areas of biomedical research, findings in Mendelian disorders started to drive transglutaminase research, particularly of FXIIIA (hemorrhagic diathesis) and transglutaminase 1 (ichthyosis), leading to basic biochemical findings, diagnostic tools and new therapies. Except TGM5 deficiency (acral peeling skin syndrome) and TG6 mutations (spinocerebellar ataxia) no inherited disease has been linked, however, to other transglutaminases. Loss of function variants in homozygous form in large human genomic data sets still have not been found for TGM2, TGM3 and TGM7 which seem to be under purifying selection in the human population. Transglutaminase knock-out mice produced in an effort to model human diseases (deletion of F13A1, TGM1), to find pathological consequences and to reveal possible physiological functions, have supplied many useful information in recent years. Notwithstanding, there are some controversies between results generated in different laboratories and not all findings could be related to humans. In case of TGM2 its deletion does not result in any apparent development or organ specific abnormality in mice while population genomics data of humans point to its possibly essential, albeit so far unknown functions. On the other hand, some observations in TGM2 KO mice (e.g. increased tissue damage to cell death inducing agents, development of autoimmune disease as a result of deficient phagocytosis of apoptotic cells, resistance to septic shock) may have relevance to human pathologies. Regarding their physiological role in humans none of the transglutaminases have been found essential for cell proliferation and maintaining cells in culture implying that their functional importance can be interpreted only in contexts of in vivo tissue functions as exemplified by specific tasks of FXIIIA in hemostasis and TG1, TG3, TG5 in skin homeostasis. Nevertheless, each transglutaminase may have differing functions at various sites in the organism as illustrated, for example, by localization of FXIII-A in macrophages or TG1 in neutrophils where it plays a significant role in the innate immune defense by stabilizing the neutrophil extracellular trap. It remains a challenge to define cellular and tissue functions of the biochemically multifunctional, in part intrinsically disordered and broadly interacting TG2 in humans; developmental defects related to cell fates (apoptosis, autophagy), its presence in high concentrations in some cell types or recent RNA-Seq data may provide clues for future studies and clarifications. Related or not to their physiological functions pathogenic circumstances may lead to inappropriate induction and/or activation of a transglutaminases playing a principle or compounding role in disease development. Most evidences have been
provided for TG2 pathologies including cataract formation, celiac disease, fibrosis, cancer, inflammation and neurodegeneration. Retinoic acid treatment of acute promyelocytic leukemia cells with therapeutic outcome often leads to potentially fatal differentiation syndrome linked to induction of high intracellular level of TG2 which boosts production and toxic release of cytokines. In neurodegenerative diseases transglutaminase catalyzed reactions could be localized to inclusions, such as Loewy bodies where cross-linked products of α-synuclein were identified and increased concentration of free ε(γ-glutamyl)lysine isodipeptide was detected in cerebrospinal fluid of patients. Some of these findings have led to clinically relevant and valuable diagnostic tools for the related disorders such as celiac disease. There are expectations that chemical inhibitors or antibodies developed recently to target extra- or intracellular TG2 may result in beneficial effects leading to novel therapies in several disease modalities.
Blood coagulation factor XIII (FXIII) is a zymogen protransglutaminase consisting of two catalytic A subunits (FXIII-A) and two carrier/inhibitory B subunits (FXIII-B). It becomes transformed into an active transglutaminase (FXIIla) in the terminal phase of coagulation cascade by the proteolytic action of thrombin and by Ca\(^{2+}\). FXIII is essential for hemostasis, it protects newly formed fibrin from the shear stress of circulating blood and from the prompt fibrinolytic degradation by cross-linking fibrin chains and α\(_2\) plasmin inhibitor to fibrin. FXIII also exerts functions outside the hemostatic system. It is required for maintaining pregnancy and is involved in wound healing. Platelets contain a huge amount of FXIII-A dimer in their cytoplasm (3% of the total platelet protein content); its intracellular activation does not need proteolysis. During platelet activation it is not secreted, but in response to strong agonist it becomes externalized. Most recently we have shown that microparticles formed during platelet activation also expose FXIII-A. Plasma FXIII and FXIII delivered by platelets are available in hemorrhagic atherosclerotic plaques and may affect cellular plaque components. We demonstrated in scratch wound and EZ4U assay that the proliferation/migration of human aortic smooth muscle cells (HAoSMCs) was significantly enhanced by FXIIla. FXIIla also increased thrombospondin secretion and matrix collagen production of HAoSMCs. These effects seem to be important in the development/maturing of atherosclerotic plaques. We published in 1985 that FXIII-A is also present in human monocytes and macrophages. Later it was demonstrated that monocytes of FXIII-A deficient patients show impaired phagocytic capacity. Reports concerning the bone marrow origin of CD34+ corneal stromal cells prompted us to look for FXIII-A containing cells in the cornea. Our finding that the normally low FXIII concentration in tears became 40-50-fold elevated after penetrating keratoplasty also raised the possibility that the source of part of this FXIII is the cornea. Indeed, a high number of stromal keratocytes showed co-staining for CD34 and FXIII-A by immunofluorescent technique. These cells were unequally distributed in the stroma, being particularly abundant in the subepithelial region. As determined by quantitative Western blotting human cornea contained 2.9 (2.1-3.7) ng FXIII-A/mg corneal protein. Active FXIII-A synthesis by stromal cells was proven by the detection of FXIII-A mRNA. Cells isolated from the stroma were characterized by flow cytometry. Corneal topography was investigated in two patients FXIII-A deficiency. Its alteration suggests that FXIII-A might play a role in keeping the structural stability of the cornea.
Macrophages are plastic cells, which respond to the changing tissue microenvironment by reprogramming their gene expression to alter their cellular phenotype and effector functions. Macrophage gene expression is primarily determined by lineage specific transcription factors (LTFs) such as PU.1, CEBP or subtype specific ones such as NFkB (classical polarization) and STAT6 (alternative polarization). In addition signal specific transcription factors (STFs) downstream of cytokine and lipid signaling also contribute to transcriptional regulation in this cell type. Our knowledge is limited regarding the hierarchy and interaction between these factors in determining macrophage gene expression and contributing to overall chromatin architecture and genome folding. In the last several years we have systematically mapped the epigenomic state of the chromatin (openness, histone marks) of non- and alternatively polarized murine macrophages. We have also determined the genome-wide localization of LTFs and STFs such as nuclear hormone receptors (RXR and PPARγ) in the same contexts. Our analyses revealed unique interactions and hierarchical relationships between these factors and unanticipated ligand independent roles for nuclear receptors in genome folding and chromatin architecture. Tissue transglutaminase appears to be one of the genes strictly and uniquely co-regulated by alternative polarization and nuclear hormone receptors and subject to epigenomic transcriptional memory and induced genome folding (looping) during repeated cytokine exposure. The global analyses of macrophage gene expression and the details of tissue transglutaminase regulation will be presented and discussed.
Factor XIII is a unique plasma based transglutaminase that requires activation by thrombin and calcium. Once activated, its main substrate is fibrin, a biological polymer with remarkable mechanical properties that supports the blood clot. Fibrin clot structure has been linked to cardiovascular disease, with clots composed of thin fibres and small pores increasing the risk of thrombosis through resistance to fibrinolysis and increased stiffness. We have investigated the effects of FXIII on clot structure and function by mutating the FXIII crosslinking sites in fibrin, thereby producing fibrin variants with altered gamma and alpha chain cross-linking. We have also investigated the effects of mutations in the FXIII activation peptide, which alter FXIII activation rates, on fibrin cross-linking and clot structure in vitro and in vivo. These studies highlight the role of fibrin cross-linking by FXIII in clot structure and function, which help to elucidate novel mechanisms that regulate thrombosis and cardiovascular disease.
THE CLINICAL SPECTRUM OF INHERITED FACTOR XIII DEFICIENCY IN THE CONTEXT OF OTHER TRANSGLUTAMINASE RELATED CLINICAL STATES
Biswa A, Singh S, Thomas A, Ivaskevicius V and Oldenburg J
Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Bonn, Germany

The first case of inherited Factor XIII deficiency was reported in a 16 year old boy from Switzerland by Duckert et al in 1960 as a severe bleeding diathesis. Since then more than 500 cases have been reported worldwide of inherited severe Factor XIII deficiency. This deficiency can manifest itself into different forms of bleeding i.e. umbilical stump bleeds, intracranial hemorrhage etc.. However, the clinical expressivity of this disorder unlike hemophilia is consistently severe and in fact more severe than other coagulative disorders. However, in the past decade the concept of inherited mild Factor XIII deficiency has come to light that is caused by a heterozygous mutation in either of the Factor XIII genes (A or B subunit). Since this form of deficiency is by and large asymptomatic unless exposed to physical trauma, its diagnosis and even clinical definition/relevance is unclear. Our group for the past decade has been involved in epidemiologically and functionally characterizing/defining this clinical state. In my talk I will present to you our findings in this regard especially in context of other known inherited Transglutaminase related disorders. I will discuss if the mild Factor XIII deficiency is a phenomenon unique to Factor XIII protein in the Transglutaminase family.
EFFECTS OF FIBRINOGEN α-CHAIN CROSS-LINKING BY FXIII ON CLOT FORMATION AND STABILITY
Duval C¹, Gold MJ¹, McPherson HR¹, Baker SR¹, Casini A², Connell SDA³ and Ariëns RAS¹
¹Thrombosis and Tissue Repair Group, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK
²Division of Angiology and Haemostasis, Faculty of Medicine, Geneva University Hospitals, Geneva, Switzerland
³Molecular and Nanoscale Physics Group, University of Leeds, Leeds, UK

BACKGROUND: FXIII plays a major role in stabilising clots by cross-linking fibrin α- and γ-chains. Previous studies using a fibrinogen mutant (γ3X) that does not support γ-chain cross-linking, indirectly revealed that α-chain cross-linking may play a role in increasing clot stiffness and decreasing clot lysis rate. We have produced a novel fibrinogen mutant (α4X) where all glutamine residues previously identified as substrate for α-chain cross-linking have been mutated.

AIMS: The aims of this study were to directly investigate the relevance of fibrin α-chain cross-linking in fibrin clot formation, structure and stability.

METHODS: Fibrinogen wild-type (WT), γ3X (γQ398N/Q399N/K406R) and α4X (αQ221N/Q237N/Q328N/Q366N) were produced in CHO cells and purified by affinity chromatography. Cross-linking of α- and γ-chains was analysed by SDS-PAGE. Fibrin lysis was studied by turbidity. Microscale fibrin viscoelasticity was analysed by magnetic tweezers.

RESULTS: Fibrinogen α- and γ-chains were cross-linked within 23 and 11 minutes (respectively) for WT, α-chain cross-linking was largely delayed (120min) for α4X, whilst γ-chain cross-linking was abolished for γ3X. Addition of FXIII increased the time to half-lysis for WT (x1.4**, but hardly for α4X (x1.2ns) and γ3X (x1.2ns). Magnetic tweezers used at 0.1Hz (clot level) and 10.0Hz (fibre level) showed that in the presence of FXIII, stiffness (G’) was significantly increased for WT (x5.1****, x2.0**) but less for α4X (x2.1**, x1.4ns) and γ3X (x1.6**, x2.5****). Non-elastic deformation (G”) was unchanged for WT and α4X, but significantly decreased for γ3X (x0.7*) at 0.1Hz. At 10.0Hz, G” was significantly increased for WT (x14.9****) and γ3X (x39.6****) but not for α4X (x1.2ns).

CONCLUSIONS: Our data show that fibrin α-chain cross-linking is a key player in reducing lysis and contributes to stiffness at single fibre and whole clot levels. This novel fibrinogen variant provides a tool for the study of (patho)physiological role of fibrin α-chain cross-linking by FXIII in-vitro and in-vivo.
PLASMA FXIII LEVELS IN HEALTHY INDIVIDUALS; REGULATION BY GENETIC AND NON GENETIC FACTORS

Katona É1, Mezei ZA1, Kállay J1, Bereczky Zs1, Kovács B1,2, Ajzner É3 and Muszbek L1
1Division of Clinical Laboratory Science, Department of Laboratory Medicine, University of Debrecen, Debrecen, Hungary; 2Borsod Abaúj Zemplén County Hospital and University Teaching Hospital, Miskolc, Hungary; 3András Jósa Szabolcs Szatmár Bereg County Hospital and University Teaching Hospital, Nyíregyháza, Hungary

**Background:** Coagulation factor XIII (FXIII) is a tetramer of two catalytic FXIII-A and two protective/inhibitory FXIII-B subunits (FXIII-A2B2). FXIII-A and FXIII-B are synthesized at different locations, they forms 1:1 complex in the plasma, where 99% of FXIII-A and ~50% of FXIII-B are present in complex. Complex formation significantly prolongs the half-life of FXIII-A in the circulation and prevents its spontaneous activation. The regulation of plasma factor XIII levels in healthy individuals has been only partially explored.


**Materials and methods:** 268 apparently healthy Hungarians were enrolled in the study. FXIII activity was measured by the ammonia release assay; FXIII-A2B2, total FXIII-B (tFXIII-B) and free FXIII-B (fFXIII-B) concentrations were measured by one-step sandwich ELISA methods; FXIII polymorphisms were determined on LightCycler 480.

**Results:** All investigated FXIII parameters showed significant positive correlation with fibrinogen level. Gender and BMI influenced only FXIII-B. FXIII activity, FXIII-A2B2 and tFXIII-B showed positive correlation with age, while there was no significant correlation between fFXIII-B and age. After adjustment to age and fibrinogen level no significant effect of current smoking on FXIII levels could be demonstrated. FXIII-A p.Val34Leu polymorphism had only slight effect on FXIII levels. In carriers of FXIII-B p.His95Arg polymorphism FXIII activity, FXIII-A2B2 and tFXIII-B levels were moderately elevated, while fFXIII-B levels were not influenced. In carriers of FXIII-B intron K C>G polymorphism all investigated FXIII parameters were significantly decreased as compared to wild type individuals and it seemed to be in synergism with the FXIII-A Leu34 allele.

**Conclusions:** Plasma FXIII levels are subjected to multifactorial regulation, in which age, fibrinogen level and FXIII-B intron K polymorphism are major determinants.
IDENTIFICATION OF THE OXIDATION SITES IN PLASMA FIBRIN-STABILIZING FACTOR (pFXIII) FOR UNDERSTANDING OF THE ALTERED PROTEIN STRUCTURE AND FUNCTION UNDER ROS ACTION

Bychkova AV\textsuperscript{1}, Vasilyeva AD\textsuperscript{1}, Indeykina MI\textsuperscript{1,2}, Bugrova AE\textsuperscript{1}, Kononikhin AS\textsuperscript{1,2,3}, Nikolaev EN\textsuperscript{1,2,3} and Rosenfeld MA\textsuperscript{1}

\textsuperscript{1}N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia
\textsuperscript{2}Moscow Institute of Physics and Technology, Russia, Dolgoprudny, Moscow region
\textsuperscript{3}Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Moscow, Russia

By applying mass-spectrometry method, the oxidation-induced modification of pFXIII has been explored. The amount of the oxidation sites revealed as well as the protein coverage depend on ROS concentration. In the oxidation process of the catalytic FXIII-A subunit among the most readily oxidizable residues are sulfur-containing amino acid residues of cysteine (positions 152, 327, 409, 695) and methionine (159, 247, 265, 350, 380, 406, 474, 475, 499, 512, 520, 595, 646, 676, and 709), cyclic amino acid residues of tyrosine (83, 103, 116, 441, 481, and 500), histidine (51, 450, 459, 605, and 666) and tryptophan (164, 292, 379, 664, and 691) of the catalytic FXIII-A subunit. For the inhibitory/carrier FXIII-B subunit there were also 6 cysteine, 4 methionine, 6 tyrosine, 5 histidine, and 3 tryptophan revealed with 1.5-2.0-fold decrease of sequence coverage compared to FXIII-A. Apparently, the abundant content of oxidized Met residues and other well-known highly-oxidized amino acid residues being ROS scavengers along with possible additional protection by FXIII-B could provide an antioxidant defense of the protein active sites that are in low-accessible areas of FXIII-A [1]. These data allow one to explain a relative functional stability of pFXIII to oxidation established earlier [2] and low stability of cFXIII subjected to induced oxidation [3]. Further studies aimed at identifying oxidation sites in pFXIII at various stages of its activation during oxidation induced by different oxidants will promote a more detailed insight into the functioning mechanism of the protein in the blood plasma undergoing oxidative stress.

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BLOOD COAGULATION FACTOR XIII IN TEARS AND ITS POSSIBLE ROLE IN CORNEAL WOUND HEALING

Orosz ZZ¹,², Szöör Á³, Veréb Z¹, Hassan Z⁴, Katona É², Vereb G³, Facskó A¹ and Muszbek L²,⁵

¹Department of Ophthalmology, University of Szeged, ²Division of Clinical Laboratory Science, Department of Laboratory Medicine, University of Debrecen, ³Department of Biophysics and Cell Biology, University of Debrecen, ⁴Orbident Health and Lasercenter, Debrecen, ⁵Vascular Biology and Thrombosis, Hemostasis Research Group of the Hungarian Academy of Sciences, University of Debrecen

Purpose. Presence of blood coagulation factor XIII (FXIII) in tears of healthy individuals was investigated and concentration changes were measured in tears of patients following corneal surgeries with different types of wound: phacoemulsification, penetrating keratoplasty (PKP) and photo-refractive keratectomy (PRK). The effect of different FXIII concentrations was also investigated on wound healing in vitro on corneal epithelial cells.

Methods. Using a hypersensitive chemiluminescent ELISA method, developed in our laboratory, FXIII complex and subunits were detected in normal tears and in tears of patients before and after different surgical interventions of the cornea, and post surgical angiogenesis and re-epithelization was observed. Scratch-wound assay, proliferation and migration assays were applied to detect the effect of FXIII on wound healing of immortalized corneal epithelial cells.

Results. Comparing to normal FXIII ranges in tears, after corneal surgeries FXIII complex and subunits concentrations increased in tears, then decreased reaching the normal interval at different times after the surgical intervention. After cataract surgery, FXIII concentrations correlated with the inflammation of the eye and the corneal oedema. Slower re-epithelisation of the corneal surface after PRK associated with lower FXIII concentrations. Extremely high FXIII concentrations measured in a few cases after PKP was associated with neovascularization of the normally avascular cornea. The addition of cellular FXIII (cFXIII) resulted in a concentration dependent faster healing of the scratch wound. cFXIII promoted the proliferation of corneal epithelial cells, but no effect on migration was observed.

Conclusion. According to our in vitro and in vivo investigations, FXIII present in tear proteome has a beneficial effect on corneal re-epithelisation, which results in decreased period of complaints caused by the corneal erosion. FXIII might be considered as an additional therapy in the treatment of corneal erosions, but long exposition to high FXIII concentrations in tears might induce undesired angiogenesis of the cornea.
TG2 modulates cell-matrix interactions and tissue stability. We have analysed the role of TG2 in four tissue injury scenarios: normal wound healing of skin and cornea, and pathological cardiac tissue remodelling as a result of chronic hypertension or myocardial infarction. 1) Closure of skin punch biopsy wounds was delayed in TG2 knockout (TG2\(^{-/-}\)) mice compared to wild-type mice. This delay was rescued with purified wild-type TG2 applied immediately post-wounding. Cell adhesion, spreading and migration studies of TG2\(^{-/-}\) murine embryonic fibroblasts (MEFs) support both an intracellular and extracellular role for TG2. 2) Corneal wound closure was also delayed in TG2\(^{-/-}\) mice compared to wild-type mice and knockdown of TG2 in corneal epithelial cells resulted in actin cytoskeleton changes that affected cell adhesion, spreading and directional migration through effects on key cell adhesion proteins in a pathway involving \(\beta-3\) integrin, paxillin, and Rac and Cdc42. 3) Hypertension was induced by transverse aortic constriction of wild-type, TG2\(^{-/-}\) and mutant TG2 knock-in mice in which the wild-type Tgm2 gene was replaced by a mutation encoding a single amino acid change that disinhibits TG2 enzyme activity, however, we observed no difference between genotypes with respect to development of cardiac hypertrophy, echocardiographic or hemodynamic parameters. 4) Myocardial infarction, induced by ligation of the left anterior descending artery of wild-type or TG2\(^{-/-}\) hearts, on the other hand, resulted in a striking increase in incidence of post-infarct ventricular free wall rupture in TG2\(^{-/-}\) vs wild-type mice in both males (100\% vs 70.6\%, \(p<0.044\)) and females (72.2\% vs 28.6\%, \(p<0.017\)), respectively, indicating a role for TG2 in modulation of post-infarct tissue injury/repair. Further characterisation of the role of TG2 in signalling pathways that promote tissue injury/repair should provide mechanistic insights that may have significant implications for the future development of safe and effective tissue repair strategies.
This presentation will look at the functional relationship between TG2 and TGFβ1 in fibrosis and in cancer. It will demonstrate the importance of this relationship at the in vitro and in vivo level in the transition of epithelial and endothelial cells to mesenchymal cells and in the transition of fibroblasts to active myofibroblasts. Using a highly selective small molecule inhibitor of TG2 the presentation will also demonstrate the therapeutic effectiveness of targeting this relationship in in vivo animal models of human disease.
TG2 INHIBITORY THERAPY IN THE TREATMENT OF FIBROTIC REMODELLING: A TRANSLATION JOURNEY

Johnson TS\textsuperscript{1,2}, Huang LH\textsuperscript{1}, Watson PF\textsuperscript{2}, Maamra M\textsuperscript{2}, Holmes T\textsuperscript{2}, Bon H\textsuperscript{1}, Brand H\textsuperscript{1}, Cain K\textsuperscript{1}, Atkinson JA\textsuperscript{1}, Wang V\textsuperscript{3}, Griffin M\textsuperscript{3} and Parr N\textsuperscript{1}

\textsuperscript{1}UCB Biopharma, Slough, UK
\textsuperscript{2}Experimental Renal Medicine, Medical School, University of Sheffield, UK
\textsuperscript{3}School of Life and Health Sciences, University of Aston, UK

Fibrotic remodelling is the largest unmet clinical need in the developed world. According to the National Institutes of Health (USA) 45\% of all deaths are directly attributable to fibrosis, with significant implications in an additional 23\%. To date only 2 anti-fibrotic pharmaceuticals are licenced, only for the treatment of IPF, with both having tolerability and efficacy issues. As such there is a demand for anti-fibrotic agents with over 75 active clinical trials in 2016.

TG2 is known to be upregulated in numerous types of fibrotic disease, with TG2 modifying studies in kidney, lung, liver and heart fibrosis suggesting a significant role in the pathobiology. It seems clear that this is an extracellular catalytic function of TG2, with direct crosslinking of ECM proteins altering turnover and ECM recruitment of the large latent TGF\textbeta\textsubscript{1} allowing activation and enhancement/stabilisation of serotonin signalling by protein incorporation appearing as central pathological roles.

To date, the lack of small molecule selective inhibitors of TG2 that have drug like properties and appropriate pharmacokinetic and pharmacodynamics has restricted clinical evaluation of TG2, although ZED1227 is reported to have appropriate specificity and the recently completed Phase1b studies should advise as to its potential. As an alternative we have developed a family of highly specific TG2 inhibitory antibodies with picomolar IC\textsubscript{50} and K\textsubscript{d} that target key epitopes in the catalytic domain.

With such high specificity, a key issue is species cross reactivity. Here we report on the generation of appropriate in vitro and in vivo translational models, including application of these antibodies to generate data sets to facilitate clinical studies. The development of suitable approaches to evaluate target engagement with applicability to demonstrating inhibition of TG2 in human target organs will be described.
Transglutaminase (TG) 3 belongs to an important family of protein cross-linking enzymes that includes nine members, three of which (TG1, TG3 and TG5) are expressed in the skin. TG3 expression is restricted almost exclusively to the differentiated granular layer of the epidermis and to hair follicles. During differentiation, TG3 undergoes proteolytic activation resulting in high cross-linking enzyme activity, indicating that TG3 is crucial for terminal keratinocyte differentiation and for the correct assembly and reinforcement of the cornified cell envelope. Here, we show that the epidermis of novel TG3-deficient mice presented mild defects in terms of skin formation, possibly due to the presence of the other skin-specific family members. Nevertheless, the skin of TG3-deficient mice demonstrated high sensitivity to the formation of cyclobutane pyrimidine dimers after UVB irradiation of the skin of newborn mice, leading to increased levels of UVB-induced cell death. The data indicated that TG3 strongly contributed to the UVB-filtering capacity of the stratum corneum by reinforcing the cornified cell envelopes adding specific cross-links. This novel finding could explain the reason for including TG3 among candidate tumor suppressor genes in human head and neck cancers.
For these years, we identified several preferred substrate sequences for Factor XIII, TG1, TG2, TG3, TG6 and TG7. Based on these sequences, highly reactive substrate peptides specifically recognized by each isozyme have been established. Fluorescence-labeled peptides appeared efficient tool to detect in situ activity of various tissue. Furthermore, these peptides are also available for identification of possible substrate proteins using biotin-labeled peptides. In this presentation, I would like to introduce the application of the peptides focusing on analyses for the keratinocyte differentiation and progression of fibrosis.

(1) In differentiating keratinocytes, both TG1 and TG3 are induced to cross-link several structural proteins, which contribute to cornified cell envelope formation essential for barrier function of the epidermis. Using the culture system, the activity and expression of TG1 and TG3 were investigated. Both enzymes were detected as an active enzyme in a different pattern at differentiation stage. Moreover, in the differentiating keratinocytes, possible TG1 substrate candidates were identified.

(2) Mouse fibrosis models for liver and kidney were produced as surgical BDL (bile duct ligation) and UUO (unilateral ureteral obstruction) procedure, respectively. In situ activities in the tissue with fibrosis progression were evaluated. Though the activities TG1 and TG2 were enhanced depending on the progression of fibrosis, the area with higher the activity were different. Furthermore, various isozyme-dependent substrate candidates in the tissue extracts were identified.

These results indicated that our peptides are available as a tool for detection of the active enzymes, and also for identification of substrates in an isozyme-specific manner.

TRANSGLUTAMINASE 2 IS A POTENTIAL REGULATOR OF SEBOCYTE MATURATION

Kovács D¹, Demény MÁ², Lovászi M¹, Zouboulis CC³, Fésüs L² and Törőcsik D¹

¹Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary ²Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary ³Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany

Transglutaminases (TGMs) are Ca²⁺-dependent acyl-transferases catalysing the posttranslational modification of various proteins. Of the eight enzymatically active isoforms, TGM1-3 and 5 are highly expressed in the human epidermis and hair follicles, with a crucial role in the terminal differentiation of keratinocytes and in the formation of the cornified envelope. In this work, we characterized the expression profiles of TGMs in the sebaceous glands of normal human skin. Immunohistochemical stainings revealed a characteristic distribution: while TGM2 was expressed most prominently in the basal cells, a diffuse expression of TGM1 and 5 proteins were detected also in the upper layers formed by maturating and dying sebocytes secreting the lipid rich sebum. Using the SZ95 sebocyte cell line, our in vitro data showed that TGM2 was the most abundant isoform of the TGMs (10 fold higher expression to TGM 1 and 5), which in line with the immunostainings, had a significant decrease in its levels with the transition from proliferation to lipid metabolism. To investigate the possible functions of TGM2 in sebocytes, we constructed TGM2 knock out SZ95 sebocytes using the CRISPR/Cas9 technique. Our results revealed that TGM2 deficiency resulted in a decreased proliferation and in an increased lipid accumulation of the sebocytes. To further understand the involved pathways and identify the molecules behind the prominent functional changes we are dissecting the differences in the gene expression profiles of the wild type vs. knock out sebocytes. Our findings suggest that TGM2 might have a key role in the differentiation of sebocytes and could serve as a potential negative regulator in their lipid metabolism.
THE DYNAMIC NATURE OF TRANSGLUTAMINASES
Hjorth-Jensen S¹, Richard D¹, Stephenson S¹, Walsh T¹ and Croll T²
¹Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia
²Cambridge Institute for Medical Research, University of Cambridge, Cambridge, CB2 0XY, UK

Introduction: Transglutaminases incorporate various primary amines into specific glutamine residues of target proteins via a transamidation reaction. Nε(γ-glutamyl)lysine crosslinks result should a lysine residue be incorporated, or alternately, biogenic amines may be incorporated to form protein-amine conjugates. While it has been suggested that transamidation may be reversible, this has never been demonstrated and it is assumed (aside from hydrolysis of products) transglutaminase activity is unidirectional. This project demonstrates transamidation is reversible and proceeds towards a dynamic equilibrium involving constant interconversion between each possible transamidated product. This implies, in a biological context, fluctuations in amine concentrations effect conversions between transamidated product varieties.

Methodology: Investigations utilised a fluorophore-labelled glutamine substrate, a quencher-labelled lysine substrate, and their fluorescently-quenched Nε(γ-glutamyl)lysine product. Transglutaminase reactions were run in the forward (from free substrates) and reverse (from product) direction, under varying concentrations and in the presence of varying amounts of competitive amines.

Results: Transglutaminase activity was shown to be freely reversible; under equivalent conditions the forward and reverse reactions converged to similar FIs at equilibrium. Furthermore, when the quenched product was incubated in the presence of histamine, the incorporated lysine substrate was exchanged with the unlabelled histamine.

Conclusions & Significance: Crosslinks may be reversibly formed or broken by transglutaminase activity as incorporated lysines are interchanged with small amines. Transglutaminases may therefore play a more dynamic role in biology than currently believed, with reversibility key to understanding many of their biological roles. This behaviour may explain why TG2-mediated serotonin incorporation is necessary for its induction of vascular smooth muscle contraction. Following an influx of serotonin, transglutaminase activity would serve to reversibly exchange stabilising Nε(γ-glutamyl)lysine crosslinks for monoamine adducts within structural/contractile proteins, resulting in destabilisation facilitative of contraction.

In conclusion, the insight provided by this project will lead to a greater understanding of transglutaminase biology and potentially lead to new avenues for disrupting disease processes.
We have identified an epidermal cancer stem cell (ECS cell) tumor subpopulation that forms rapidly growing and highly invasive and vascularized tumors. TG2, VEGF-A and neurophilin 1 (NRP-1) are markedly overexpressed in ECS cells, and are required for ECS cell survival and tumor growth/vascularization. VEGF-A/NRP-1 interacts with a TG2/α6/β4-integrin complex to activate FAK/Src signaling which leads to stabilization of YAP1/ΔNp63α to drive ECS cell survival, and tumor growth and angiogenesis. Loss of TG2 markedly reduces ECS cell spheroid formation, invasion and migration, and pharmacologic inactivation of TG2 results in dramatic reduction in tumor growth and vascularization. These studies suggest that TG2 is a key component of a pro-angiogenic complex and is required for tumor growth and angiogenesis.
Cancer is a highly complex disease displaying marked inter- and intra-tumoral heterogeneity. Although most cancers respond to therapies initially, in many patients the disease reappears in form of metastases (recurrent tumors). Metastatic tumors are generally resistant to therapies (refractory cancer), as a result the patients succumb to the disease due to uncontrolled growth of resistant cancer. Recent discoveries have supported the presence of a small subpopulation of self-renewing cancer stem cells (CSCs) in tumors that are inherently resistant and survive toxic effect of therapy. Based on this conviction, it is now believed that eradication of CSCs is critical for complete ablation of cancer.

Many reports have identified transglutaminase II (TG2) as among the most abundant overexpressed proteins in multiple drug-resistant and metastatic tumors and tumor cells lines. This is an important observation as increased expression of TG2 in tumor samples from cancer patients is associated with shorter remissions and poor survival rates. Chronic expression of TG2 in cancer cells led to the activation of a developmental program of epithelial to mesenchymal transition (EMT) and stem cell traits, the two important hallmarks of metastatic cancer.

At molecular level, TG2 expression constitutively activated the nuclear transcription factor (NF)-kappa B via a non-canonical pathway. Hypoxia-induced factor (HIF)-1alpha was identified as one of the downstream targets of TG2-induced NF-kappaB. TG2-dependent activation of HIF-1alpha, resulted in increased glucose uptake and altered metabolism from oxidative to aerobic glycolysis. Conversely, experimental suppression of TG2 attenuated the invasion and cell motility function and sensitized cancer cells to chemotherapeutic drugs.

Many recent studies have supported that ‘compact or closed’ conformation of TG2 is essential for oncogenic signaling while ‘open or extended conformation’ acts as proapoptotic factor. Taken together, these results suggest that targeting of TG2 could offer a promising therapeutic option for eradicating CSCs and treating refractory/recurrent tumors. However, due to complex structure and multifunctional nature of TG2, it is challenge to design small molecule inhibitors to target it. To overcome this challenge, we used gene-specific small interfering RNA (siRNA) for targeting TG2. Although siRNA is a validated method for silencing gene expression, its delivery due to susceptibility to breakdown in vivo, has been limited to site-specific injections, which is not always practical in the clinic. As protective carriers of siRNA we developed liposomes, composed of synthetic lipids to deliver siRNA to in vivo growing tumors. Liposomemediated delivery of TG2-siRNA downregulated TG2 expression and inhibited the growth...
and dissemination of ovarian and pancreatic tumors in a mouse model. We believe that discovery of TG2 as a therapeutic target offers a great promise to address an important unmet medical need of treating recurrent and refractory tumors. Currently, we are working on preclinical development of a safe and affordable nanosomal-TG2 siRNA formulation to harness TG2 expression in cancer patients.
INTRACELLULAR FACTOR XIII IS A PROGNOSTIC MARKER IN PEDIATRIC LYMPHOBLASTIC LEUKEMIA

Kappelmayer J¹, Hevessy Z¹, Kárai B¹, Szánthó É¹, Katona É², Gyurina K³, Szegedi I³ and Kiss C³

¹Department of Laboratory Medicine, ²Division of Clinical Laboratory Science, ³Department of Pediatrics, Faculty of Medicine, University of Debrecen, Hungary.

Background: The ‘A’ subunit of the plasma transglutaminase coagulation factor XIII (FXIII-A) is present intracellularly in megakaryocytic and monocytic cell lines. In earlier studies we have identified FXIII-A as a more sensitive marker of acute myeloblastic leukemias (FAB M4, M5, M7) than conventionally used surface markers.

Purpose of study: Since in the investigation of acute leukemias the identification of a leukemia associated immunophenotype (LAIP) is considered important, we investigated whether FXIII-A can be regarded as a LAIP in blasts of acute lymphoblastic leukemias (ALL).

Methods: Magnetically separated normal B-cell precursors and bone marrow samples from ALL cases, were investigated by 4-8 color immunophenotyping by flow cytometry. FXIII-A was also detected by confocal laser scanning microscopy, and lysates of normal and leukemic B-cells were analysed by Western-blot and ELISA techniques.

Results: In lymphoid blasts derived from de novo cases suffering from ALL we detected FXIII-A by a directly conjugated antibody in 67% of pediatric ALL cases. The presence of FXIII-A was verified as an 82 kD band under reducing conditions in lysates of ALL blasts but it was consistently negative in healthy B-cells, normal B-cell precursors and in B-cells from chronic lymphoid leukemia cases. The 10-year event-free survival (EFS) and overall survival (OS) rate of FXIII-A-positive and FXIII-A-negative patients showed significant differences (EFS: 84% vs. 61%, respectively; p = 0.031; OS: 89% vs. 61%; p = 0.008). In the FXIII-A positive cases reduction of blast percentage was significantly higher in the FXIII-A positive blast subclone than in the FXIII-A negative one both in day 15 (p=0.0003) and in day 33 (p<0.0001) bone marrow aspirate samples.

Conclusion: We found FXIII-A positivity as a LAIP in pediatric ALL that provides an excellent correlation with survival. The results suggest a possible role for FXIII-A expression in the prognostic grouping of childhood BCP-ALL patients.
ROLE OF TRANSGLUTAMINASE 2 IN SELECTIVE DELETION OF MYCN+CD133+ LIVER CANCER STEM CELLS BY ACYCLIC RETINOID

Qin XY¹, Shrestha R¹, Sekihara S¹, Wada A² and Kojima S¹
¹Micro-Signaling Regulation Technology Unit, RIKEN Center for Life Science Technologies, Wako, Japan
²Department of Organic Chemistry for Life Science, Kobe Pharmaceutical University, Kobe, Japan

Hepatocellular carcinoma (HCC) is a highly lethal cancer that has a high rate of recurrence in part because of cancer stem cell (CSC)-dependent “field cancerization”. Acyclic retinoid (ACR) is a synthetic vitamin A–like compound capable of preventing the recurrence of HCC. We previously reported that ACR induced nuclear translocation of the crosslinking enzyme transglutaminase 2 (TG2) by accelerating the formation of a trimeric complex with importin-α/β. Subsequently, accumulated TG2 in nucleus resulted in crosslinking and inactivation of Sp1 transcription factor, thereby reducing expression of the Sp1-responsive genes such as epidermal growth factor receptor (EGFR) thereby apoptosis of hepatic cancer (stem) cells. In contrast, suppression of TG2 partially restored these phenomena. Here, we performed a genome-wide transcriptome screen and showed that ACR selectively suppressed the expression of MYCN in HCC cell cultures but not normal hepatic cells. In addition, high-content single-cell imaging analysis showed that MYCN+CD133+CSCs in the heterogeneous HCC cell cultures were selectively killed by ACR and cooperatively deleted following MYCN knockdown. Further pathway analysis suggested a TG2/Sp1/MYCN/caspase-8 signaling pathway underlying the ACR’s effect. Binding of purified TG2 to an NH₂ derivative of ACR immobilized FG beads was observed and confirmed using competition binding assay with free ACR. Notably, Sp1 has been reported to cooperate with E2F in activating the MYCN promoter. Along with this line, a gene-selective Sp1 inhibitor, Mithramycin A, significantly inhibited MYCN expression in HCC cell cultures. Upon knockdown of MYCN, HCC cells showed suppressed cell growth and increased caspase-8 activity, whereas a caspase-8 inhibitor z-IETD-FMK restored ACR-induced growth suppression. In summary, our data suggested MYCN as a therapeutic target of ACR in selective deletion of liver CSCs. Furthermore, ACR binds to TG2 and prevents hepatic tumorigenesis through a TG2/Sp1/MYCN/caspase-8 dependent signaling pathway.
Transglutaminase 2 (TGase 2) represents an interesting target for the development of PET tracers for functional imaging of tumours. Among the TGase 2 inhibitors described in the literature, \(N^\alpha\)-phenylacetyl-\(N^\varepsilon\)-acryloyl-L-lysine-4-(6-methylpyridin-2-yl)piperazide (1) \[1\] seems to be most suitable for radiotracer development as this compound exhibits both strong inhibitory potential and selectivity towards human (h) TGase 2. Extensive structure-activity relationship studies by our group revealed some potent fluorinated analogues of 1, of which compound 3 (methyl group is substituted by fluorine) is a potential candidate for PET tracer development due to its great inhibitory potency and promising pharmacokinetic properties.

For the radiosyntheses of \([^{18}\text{F}]3\), 6-nitro (2a) and 6-trimethylammonio-pyridines (2b) were envisaged as precursors for direct \(^{18}\text{F}\)-fluorination. The fluorination reactions using \([^{18}\text{F}]\)fluoride were performed under various conditions. Labelling of 2a by \([^{18}\text{F}]\)fluoride resulted in only moderate radiochemical yields (\(\approx 20\%\)) accompanied by the formation of two \(^{18}\text{F}\)-labelled side products. In contrast to this, \(^{18}\text{F}\)-fluorination of 2b yielded exclusively \([^{18}\text{F}]3\) in high radiochemical yields (\(\approx 70\%\)). Therefore, precursor 2b was chosen for further radiosyntheses. In vitro characterisation of \([^{18}\text{F}]3\) with regards to its reactivity towards hTGase 2 as well as its selectivity and specificity was done by radio-TLC and radio-SDS-PAGE. Kinetic investigations by radio-TLC provided values for \(k_{\text{inact}}/K_1\) that are in good agreement with values obtained by fluorimetric activity assays. Incubation of whole cell lysates of different human tumour cell lines exhibiting a high expression of TGase 2 with \([^{18}\text{F}]3\), followed by SDS-PAGE and measurement of the \(^{18}\text{F}\) activity revealed essentially a single band around the molecular mass of hTGase 2 (\(\approx 77\ \text{kDa}\)). Accordingly, no band was observed for those tumour cells which do not express TGase 2. Further experiments with \([^{18}\text{F}]3\) will include cell uptake studies in living tumour cells as well as stability, biodistribution and PET studies in mice.

DELETION OF ASTROCYTIC TRANSGLUTAMINASE 2 IMPROVES OUTCOMES SUBSEQUENT TO INJURY
Monteagudo A¹, Proschel C³, Feola J¹, Gil de Rubio R¹, Natola H³, Nadtochiy S² and Brookes PS¹,² and Munger J⁴ and Johnson GVW¹,²

Departments of ¹Pharmacology and Physiology, ²Anesthesiology, ³Molecular Genetics, and ⁴Biochemistry, University of Rochester, Rochester, NY, USA

In response to central nervous system (CNS) injury, astrocytes take on what is called a reactive phenotype, and play either beneficial or detrimental roles depending on the type of CNS injury and the different molecular programs initiated. Identifying and understanding the role modulatory proteins play in directing the different molecular programs following injury is essential. It is becoming clear that transglutaminase 2 (TG2) is one of the proteins that play key roles in regulating astrocyte function and response to injury. Previously we have shown that global deletion of TG2 results in positive outcomes following ischemic brain injury in vivo. Studies in primary astrocyte cultures have also implicated TG2 as a negative modulator of astrocyte viability subsequent to injury. We have now extended these findings and show that knocking down TG2 in astrocytes significantly increases their ability to protect neurons in a transwell model of oxygen glucose deprivation/reperfusion (OGD/reperfusion) injury. Further, our findings suggest that deletion of TG2 in astrocytes results in a more favorable metabolic outcome subsequent to injury (OGD/reperfusion), which could contribute to their resistance to ischemic-induced cell death and increased ability to support neuronal cell health. We extended these findings into a mouse model where we deleted TG2 just in astrocytes to determine how the absence of astrocytic TG2 facilitates functional recovery after CNS injury. Our results show that, subsequent to a spinal cord injury, mice with TG2 deleted just in astrocytes recover mobility to a significantly greater extent than mice expressing normal TG2 levels. These and other findings indicate that TG2 plays a key role in mediating astrocyte function following CNS injury.
Transglutaminase is present in the brain and has been thought to participate in the protein aggregation characteristic of neurological diseases such as Huntington, Alzheimer’s and Parkinson’s disease. We have developed a functional proteomics strategy in which biotinylated amine-donor and amine-acceptor probes were used to identify the transglutaminase substrates present in brain. Bioinformatics analyses revealed that most of the 166 brain substrates identified interacted with huntingtin, the amyloid precursor protein or α-synuclein and that neurological disease was the most significant canonical pathway associated with the substrates. The physiological relevance of the substrates identified by mass spectrometry was confirmed by the fact that three of the substrates (actin, β-tubulin and a neurofilament subunit) were polymerized in neuronal cells when cytosolic calcium concentration was raised. We also showed by in-situ immunolabeling that some of the substrates were part of the protein aggregates found in neurological diseases. These results strongly support the idea that the crosslinking activity of brain transglutaminase participates in the formation of the protein aggregates found in diseases of the central nervous system.
Multiple Sclerosis (MS) is a chronic neuroinflammatory disease, clinically resulting in sensory and motor impairment, and cognitive deficits. Neuropathological characteristics of MS are alterations in blood-brain barrier function, perivascular infiltration and migration of leukocytes, activation of local glial cells, demyelination and axonal loss.

Tissue Transglutaminase (TG2) is a multifunctional enzyme, well known for its role in cross-linking of proteins. In addition, TG2 acts as a co-receptor for β-integrins to enhance fibronectin binding. In this way TG2 is considered to contribute to cell adhesion and migration process. In a previous study we observed the appearance of TG2 in MHCII positive cells in active MS lesions. Moreover, in an experimental model for MS, TG2 mediated the infiltration of monocytes into the CNS and inhibition of TG2 activity dramatically reduced clinical symptoms.

These data prompted us to study TG2 in monocytes from MS patients with ongoing disease. Thus, in the present study we questioned whether TG2 expression is altered in monocytes from MS patients compared to control subjects? Moreover, we determined the inflammatory status of the TG2 expression cells and whether TG2 can functionally contribute to human monocyte adhesion/migration processes.

We observed that TG2 mRNA levels were significantly increased in monocytes derived from MS patients. In addition, correlation analyses indicated that TG2-expressing cells from MS patients display a more anti-inflammatory, migratory profile. Using the human THP1 monocytic cell-line, we observed that interleukin-4 (IL-4) was a major trigger of TG2 expression in this cell type. Furthermore, knock-down of TG2 expression led to a pro-inflammatory profile and reduced adhesion/migration of IL-4 treated monocytes. We now propose that IL-4 can be an important enhancer of TG2 expression in monocytes of MS patients, driving them into an anti-inflammatory status. Functionally, TG2 could, at least partly, mediate the enhanced adhesion of anti-inflammatory monocytes to the CNS endothelium of MS patients.

Van Strien ME et al., Brain Behaviour and Immunity 50:141-154, 2015
Type 2 transglutaminase (TG2) is a calcium-dependent protein cross-linking enzyme activated in mis-folding diseases (e.g. Alzheimer’s disease). In vitro TG2 has been implicated in the generation of toxic Aβ oligomers, and Aβ and tau are substrates of TG2-mediated transamidation in brain. Although TG2 may be directly involved in the progression of neurodegeneration, the underlying mechanism remains unclear. Here we have characterised TG2 in primary rat embryonic hippocampal neurons exposed to Aβ, to mimic a neurodegenerative environment. Dual immunofluorescence staining of TG2, and either presynaptic marker vGlut1 or postsynaptic marker Shank2 revealed that TG2 is mainly located at neuronal pre-synapses. Stimulus with Aβ induced a 6-fold increase and spread of TG2 activity in situ, when visualised by incorporation of fluorescent primary amine substrate FITC-cadaverine into endogenous γ-glutamylsubstrates. To identify potential substrates of TG2-transamidation (“TG2 transamidome”) we employed a global quantitative proteomic approach involving incubation of hippocampal neurons with FITC-cadaverine either in the presence of Aβ, or of Aβ with a TG2 inhibitor. Proteins cross-linked to FITC-cadaverine were immunoprecipitated (IP) from total cell lysates with an anti-FITC antibody, proteolytically digested and analysed via SWATH™-MS/MS proteomics (n=3) using a spectral library produced by classic shotgun-MS. Differences between Aβ and Aβ-TG2 inhibitor (representing the background) were identified at a confidence ≥70%. Furthermore, the proteome of hippocampal neurons with and without Aβ treatment was also resolved, with a coverage of 1000+, and interrogated with the “TG2 transamidome”. Six proteins were found to be specific substrates of TG2-transamidation upon Aβ. An ionic channel protein was a highly specific substrate of TG2 and its involvement in the modulation of calcium homeostasis at neuronal synapses, and thus further TG2 activity, is currently being investigated.
AUTOIMMUNITY TO TRANSGLUTAMINASE 2 IN CELIAC DISEASE
Șollid LM$^{1,2}$, Blazevski J$^1$, du Pré MF$^1$, Iversen R$^1$, Lundin KEA$^3$, Neumann RS$^2$, Qiao SW$^{1,2}$, Roy B$^1$, Sandve GK$^3$, Snir O$^1$ and Stamnaes J$^1$

$^1$Centre for Immune Regulation, Department of Immunology, University of Oslo and Oslo University Hospital; $^2$KG Jebsen Coeliac Disease Research Centre, Department of Immunology, University of Oslo, Oslo, Norway; $^3$KG Jebsen Coeliac Disease Research Centre, Department of Gastroenterology, University of Oslo and Oslo University Hospital, Oslo, Norway; $^4$KG Jebsen Coeliac Disease Research Centre, Department of Informatics, University of Oslo, Oslo, Norway.

Celiac disease is characterized by IgA and IgG autoantibodies directed to transglutaminase 2 (TG2). Increased levels of anti-TG2 antibodies in serum with the revised diagnostic criteria can be diagnostic in children. Anti-TG2 autoantibodies only develop in subjects who carry the HLA allotypes DQ2 or DQ8 when they are consuming cereal gluten proteins. To understand why anti-TG2 autoimmunity develops in response to an exogenous environmental factor, we have characterized the immunoglobulin antigen receptors of thousands of TG2-specific plasma cells of celiac disease lesions. Results from this endeavor will be presented. We have also developed an immunoglobulin knock-in mouse based on the rearranged immunoglobulin heavy and light chain genes of a celiac lesion derived single plasma cell that encode an antibody recognizing both human and mouse TG2. Preliminary results from the mouse model of anti-TG2 autoimmunity will also be presented.
ROLE OF TRANSGLUTAMINASE 2 EPITOPES IN THE SELECTIVE DIAGNOSIS OF CELIAC DISEASE

Korponay-Szabó IR
Dept. of Pediatrics, Faculty of Medicine, University of Debrecen, Debrecen and Heim Pál Children’s Hospital, Budapest, Hungary

Celiac disease is characterized by the development of small intestinal villous atrophy and malabsorption in response to cereal gluten ingestion. Gluten-induced autoantibodies against transglutaminase 2 (TG2) target specific surface parts of TG2 and influence the enzymatic activities of the protein. It is currently, however, unclear why the same TG2 epitopes are the preferential sites of the reaction in different patients and why the two main epitope groups are found on opposite surfaces of TG2. Further, little is known on the pathogenetic role of the autoantibodies, although it is clear from the clinical experience that they are invariably induced in all celiac cases and do bind to the tissues in vivo. It appears that tissue binding is skewed according to TG2 epitope availability determined by fibronectin and collagen interacting partners and during the remodelling of gut mucosa it changes towards the compensatory expansion of tissue compartments with less TG2. Interestingly, only vessel walls and specialized fibers (reticulin) contain extracellularly accessible TG2 but the normal connective tissues do not. Utilizing epitope-specific quantitation of patient antibodies it is possible to investigate changes in antibody composition also in the blood and correlate the findings with clinical prediction tools. A recent prospective multicenter European study (Werkstetter, Gastroenterology in press) confirmed that high serum TG2 antibody levels by certain clinical tests reliably predict celiac disease and serum antibody determination can replace duodenum biopsy in clinical practice. However, for the correct measurement of antibody content and interchangeable results, relative (arbitrary) units used in current clinical tests should be replaced by exact concentration determinations. One option to circumvent analytical challenges in measuring polyclonal antibodies is to construct epitope-specific determination arrays based on selective TG2 mutants which at the same time will distinguish celiac antibodies from anti-TG2 antibodies in other diseases and thus ensure high clinical specificity.
Accumulation of TG2 in the nucleus induces apoptosis in both normal hepatic cells treated with alcohol/free fatty acids (Gastroenterol 2009; JCP 2012) and hepatic cancer cells treated with acyclic retinoid (ACR) (Mol Cancer 2011) via crosslinking and silencing Sp1 transcription factor thus decreasing expression of growth factor receptors. ACR directly binds to TG2 and enhances complex formation with importins α/β, therefore getting localized in the nucleus (CDDIS 2015; Qin et al. this symposium). Here, we studied the impact of fungi/bacteria on nuclear TG2 activation and the subsequent hepatic cell death (Sci Rep in press).

The liver acts as the first barrier to the spread of fungi and bacteria present in intestine. In ASH and NASH patients, these fungi and bacteria invade gastrointestinal mucosa to reach the liver. Co-incubation of either human hepatic cells or mouse primary hepatocytes derived from wild-type but not TG2−/− mice with pathogenic Candida species and E. coli, but not Saccharomyces cerevisiae, induced cell death in host cells by enhancing nuclear TG activity. This phenomenon was mediated partly by reactive oxygen species (ROS) such as hydroxyl radicals, as detected by a fluorescent probe and ESR. Both N-acetyl cysteine (a ROS scavenger) and phenosafranin (an inhibitor of nuclear localization of TG2) suppressed nuclear TG activity and inhibited cell death, while deletion of C. glabrata nox-1, which encodes a ROS-generating enzyme, resulted in a strain that failed to induce the same phenomena. A similar induction of hepatic ROS and TG activities was observed in C. albicans-infected mice.

These results address for the first time, an association of ROS-producing fungi like C. albicans and glabrata as well as E. coli with enhanced nuclear TG2 activity in hepatic cells leading to apoptosis, which may explain a molecular mechanism of hepatic injury seen in ASH/NASH patients.
The term Proteostasis was coined to represent the functional balance of the proteome, which is permanently challenged by environmental stress and alterations in physiology. A decline in proteostasis leads to progressive aggregation of misfolded proteins, overwhelming the cellular protein quality control networks. Proteostasis depends on constant protein degradation and resynthesis, and is essential for proper homeostasis in systems from single cells to whole organisms. Cells possess several mechanisms and processes to maintain proteostasis. The heat shock proteins modulate protein folding and repair in case this process fails the proteasome and autophagy as well as other lysosome-dependent systems, function in the degradation of dysfunctional proteins. In the last few years we have been studying how these systems interact to maintain cellular proteostasis. We focused on Transglutaminase 2 (TG2), a multifunctional enzyme, which is deregulated in several human diseases that are characterized by unbalanced cellular adaptation to cell-autonomous or environmental stress leading to deficient proteostasis. First we showed that TG2 plays a key role in the regulation of the final steps of autophagy. More recently we demonstrated that the trimerization and activation of heat shock factor 1 (HSF1), the main transcriptional factor of the stress-responsive genes, is mediated by TG2. TG2 regulates adaptation to stress and proteostasis by acting as a protein disulphide isomerase (PDI). We find that TG2 ablation is correlated to a defect in the nuclear translocation of HSF1 and in the DNA binding ability to HSP70 promoter. In vivo and in vitro heat shock treatments show that the expression of the stress inducible chaperone HSP70 is dependent on TG2. Notably, we show that TG2 inhibition of its PDI activity with cysteamine restores the unbalance in HSF1-HSP70 pathway in Cystic fibrosis, a human disorder characterized by deregulation of proteostasis. Altogether, these results indicate that TG2 plays a key role in the regulating of cellular proteostasis through the modulation of the heat shock response.
LOSS OF TRANSGLUTAMINASE 2 SENSITIZES FOR THE DEVELOPMENT OF INFLAMMATION AND INSULIN SENSITIVITY IN MICE KEPT ON HIGH FAT DIET

Köröskényi K1, Sághy T1, Miklós A2, Hegedűs K2 and Szondy Z1

1Department of Biochemistry and Molecular Biology, and 2Department of Anatomy University of Debrecen, Debrecen, Hungary

Transglutaminase 2 (TG2) is a multifunctional enzyme with many biological functions. One of its prominent functions is to ensure effective clearance of apoptotic cells. Increasing amount of evidence indicate that defective clearance of dying cells can lead to the development of chronic systemic inflammatory diseases, and loss of TG2 seems to sensitize for various chronic inflammatory diseases, such as atherosclerosis and autoimmunity. Obesity is also characterized by chronic low-grade inflammation and by accumulation of apoptotic cells and inflammatory macrophages in the adipose tissue. It is recognized that this chronic inflammatory state is involved in the pathogenesis of obesity-related insulin resistance, metabolic syndrome and type 2 diabetes.

In this study, we investigated the effects of the loss of TG2 and thus impaired clearance of apoptotic cells on the development of obesity, insulin resistance and obesity related inflammation. We performed a 17-week long feeding experiment in which TG2 KO mice and their wild type counterparts were fed with either low or high fat diet. Our preliminary results indicate that TG2 deficient mice - kept on low or on high fat diet - are characterized by enhanced apoptotic cell accumulation in the adipose tissue, and enhanced insulin, adipokine and macrophage derived inflammatory cytokine production as well as prediabetic insulin resistance as compared to their wild type counterparts. Our data describe a new phenotype of transglutaminase 2 null mice.

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BROWNING DEFICIENCY AND LOW MOBILIZATION OF TRIACYLGLICEROLS IN GONADAL WHITE ADIPOSE TISSUE LEADS TO DECREASED COLD-TOLERANCE OF TRANSGLUTAMINASE 2 KNOCK-OUT MICE

Mádi A1,2, Monroy IC1, Lénárt K1, Pap A1, Kristóf E1, Oláh A3, Vámosi G4, Bacso Z4, Bai P5 and Fésüs L1,2

1Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary
2MTA-DE Stem Cells, Apoptosis and Genomics Research Group of the Hungarian Academy of Sciences, Debrecen, Hungary
3Department of Laboratory Medicine, University of Debrecen, Debrecen, Hungary
4Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary
5MTA-DE Lendület Laboratory of Cellular Metabolism Research Group, Research Center for Molecular Medicine, Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary

When energy intake chronically exceeds, white adipose tissue (WAT) expands leading to obesity and associated diseases. The less abundant thermogenic brown AT (BAT) is responsible for the maintenance of body core temperature. Interestingly, during cold-exposure 'beige' adipocytes appear within WAT supporting the tolerance of the organism. These cells, similarly to BAT, dissipate stored chemical energy in the form of heat, thus, increasing energy expenditure. Exploration of this browning process of WAT may lead to new strategies for treatment of obesity and related disorders. We have studied the effect of TG2 on AT formation in the mouse model. Although TG2+/+ and TG2−/− mice have the same amount of AT, we have found that TG2+/+ animals can tolerate acute cold exposure for 4h while TG2−/− mice only for 3h. TG2−/− and TG2+/+ animals have used up half of their subcutaneous AT (SCAT) after 3h treatment; however, TG2−/− mice still possess markedly whiter and higher amount of gonadal AT (GONAT) compared to TG2+/+ animals (258+/−28 mg vs. 172+/−16 mg). Limited utilization of GONAT in TG2−/− mice has been reflected in the significantly larger size of adipocytes (479+/−51 µm² vs. 296+/−14 µm²) and lower free fatty acid levels in sera (1.28+/−0.12 mM vs. 2.12+/−0.1 mM). Moreover, we have measured significantly lower expression of ‘beige’ marker genes in TG2−/− GONAT, such as UCP1 (0.236+/−0.11 FC), TBX1 (0.357+/−0.086 FC) and TNFRFS9 (0.416+/−0.174 FC) compared to TG2+/+ samples after 3h cold exposure. We have confirmed the significantly lower expression of UCP1 at protein level in cold-treated TG2−/− GONAT using Western blot and immunohistochemistry. This mitochondrial inner membrane carrier dissipates protonmotive force and increases the rate of substrate oxidation to generate heat. Our data suggest that
TG2 has a crucial tissue-specific role in development of GONAT that comes into apparent under stressful conditions like acute cold exposure.
Acute promielocytic leukaemia (APL) NB4 cells can be differentiated to neutrophil granulocytes by all-trans-retinoic-acid (ATRA) treatment used as a differentiation therapy in patient with APL to result in terminal differentiation of promielocytic cells. This method results in up- and down regulation of several genes to generate functional neutrophil granulocytes with abilities for quick immune responses. One of the most up-regulated genes in ATRA induced maturation of NB4 cells is transglutaminase 2 (TG2). Silencing of TG2 expression in NB4 cells revealed functional contribution of TG2 to adhesion, migratory, phagocytic capacity of neutrophils and superoxide (ROS) production (Balajthy Z, et al. Blood. 2006; Csomós K, et al. Blood. 2010).

Retinoic acid syndrome or differentiation syndrome (DS) is a severe complication associated with the treatment of APL with ATRA, in which induction and secretion of CC chemokines (CCL2/MCP-1, CCL22/MDC, CCL23/MIP-3, CCL24/MPEF-2) and cytokines result in neutrophil infiltration into tissues (lungs and heart). Knocking down TG2 expression in ATRA differentiated NB4 cells suppressed both chemokines/cytokines and ROS production suggesting a role of TG2 in triggering DS (Csomós K, et al. Blood. 2010).

ATRA differentiated NB4 cells may act as inflammatory neutrophils. Several genes like gpPhox91, CCL2/MCP-1 and others are regulated through the NF-κB pathway. To figure out the function of both TG2 and NF-κB in this pro-inflammatory state of differentiated NB4 cells we knock out either the TG2 with the technology of TALEn (Transcription activator like effector nucleases) to estimate NF-κB transcription activity in differentiated NB4 TG2-KO cell lines followed the generating a new NF-κB sensor NB4 TG2-KO sub-cell lines expressing NK-κB transcriptional response element regulated reporter (luciferase) genes.

Functional characterization of NB4 TG2-KO cells and the contribution of transcriptional activity of NF-κB to the development of DS in differentiated NB4 cell lines will be presented.
 TARGETED COVALENT INHIBITION OF TISSUE TRANSGLUTAMINASE IN CANCER STEM CELLS
Keillor JW$^1$, Akbar A$^1$, McNeil NMR$^1$, Kerr C$^2$ and Eckert RL$^2$

$^1$University of Ottawa
$^2$University of Maryland

Recent evidence has shown that tissue transglutaminase (TG2) is a key player in the metastatic progression of cancer. TG2 is an enzyme that was first identified for its ability to mediate the extracellular cross-linking of proteins through the formation of isopeptide bonds; this transamidation reaction is catalysed by the enzyme when it adopts an extended conformation. However, TG2 is also known to play a role as a GTP-binding protein in intracellular signalling, when it adopts a dramatically more compact conformation. Most recently, TG2 expression has been implicated in inducing the epithelial-mesenchymal transition (EMT) in ovarian, breast and epidermal cancer stem cells, contributing to their development of drug resistance and metastatic competence.

Over the past ~20 years of research into TG2 mechanism and inhibition, we have developed many reversible and irreversible inhibitors. Recent results will be presented, where we have demonstrated that our targeted covalent inhibitors are able to modulate the conformation of TG2 and abolish both transamidation and GTP-binding activities, in vitro and in living human cells. Furthermore, collaborative efforts have shown that several of our inhibitors are able to halt the EMT of epidermal cancer stem cells (CSCs) and reduce tumour growth through a mechanism of action that involves perturbation of a specific signalling pathway critical to CSC survival.
CovIsoLink™: NOVEL SITE-SPECIFIC ADCs GENERATION USING mTG CROSSLINKING

Sivado E1,2, Thomas V1, El Alaoui M2, Dyson MR3, Jones P3, McCafferty J3, Valsesia-Wittmann S2 and El Alaoui S*1

1 Covalab, 11 Bd Einstein 69100 Villeurbanne, France,
2 Centre Léon Bérard, Immunotherapy lab-P3I, 28 rue Laennec 69008 LYON, France,
3 IONTAS Ltd, Unit 2, Iconix Park, Pampisford Cambridge UK

*elalaoui@covalab.com

CovIsoLink™ (Covalently Isopeptide crosslinking) relates to methods for enzymatic covalently coupling drugs and other compounds through transglutaminase site specific generated in the targeted proteins including, polypeptides, proteins and immunoglobulins (patent pending1). Transglutaminases (TGases) catalyze covalent cross-linking of specific glutamine residues to the primary amine of peptide-bound lysine residues or primary amines of other compounds such as polyamines2. Using an in house peptide library, and a transglutaminase colorometric assay3, we developed a screening system to identify primary structures surrounding reactive glutamine residues that are preferred by TGases. Screening was performed by selecting peptides that incorporated biotin-labelled primary amine (biotin cadaverine) by the catalytic reactions of microbial and tissue transglutaminases (mTG, tTG). We identified several small peptides that were recognized as glutamine donor substrates. The optimum peptide sequences were selected and further confirmed that these sequences have improved affinity compared with the known peptides (such as ZQG, ...).

Monoclonal antibodies coupled to highly toxic agents or ADC (Antibody-Drug Conjugate) are becoming a significant component of anticancer treatment. ADCs are a new class of powerful drugs designed to target high-dose chemotherapy directly to cancer cells. Trastuzumab/emtansine (T-DM1 Kadcyla R) is an ADC that has been shown to cause significant. Despite their growing success, commercial ADCs are still heterogeneous mixtures. They are largely manufactured using chemical conjugation methods, in which the cytotoxic drug is covalently attached to lysines or cysteines on the antibody. These methods generally result various number of drugs (0 to 8) attached to different positions on the antibody determining the DAR (drug-antibody ratio). At present, the DAR distribution is not fully controlled, while it influences the pharmacokinetic-pharmacodynamic profiles of ADCs: the naked antibody (DAR0) is a competitive inhibitor, ADCs with a low DAR display poor efficiency, and those with a high DAR are rapidly eliminated in plasma. Depending on the payload, DAR2 seems to be the best compromises.
CovIsoLink™ is used to develop new ADCs since the major advantage of this method is to obtain a homogenous immunoconjugate with uniform stoichiometry by controlling: (a) the location of coupling sites on the antibody without affecting its immunoreactivity and (b) the number of molecules coupled per molecule of antibody by controlling the DAR and consequently the toxicity and efficacy of therapeutic molecules.

We will discuss the results of our ADC in comparison with Trastuzumab-emtansine (T-DM1) targeting Her2/neu, using series of *in vitro* and *in vivo* models.

Transglutaminase (TG) 2 is an enzyme with a predominant role in the cell stress response and in tissue repair. TG2 is released from cells via an unconventional secretion pathway, and this mechanism controls extracellular activity. This pathway is likely to be shared with other proteins undergoing alternative secretion, many of which are potent biological signals, and hence understanding the regulatory mechanism for this pathway is of central importance.

Purinergic (P2X) receptors are ion channels with important roles in innate immunity. Extracellular ATP released from compromised tissue/cells triggers their activation, and P2X7R activation can act as a danger signal amplification system within the local milieu through paracrine signalling. We have shown that rapid active export of TG2 is linked to activation of P2X7R, and that through co-secretion of thioredoxin-1, this mechanism controls extracellular levels of active enzyme (1). Our present aim is to gain an understanding, at the molecular level, how P2X7R activation couples to downstream events including TG2 externalization and inflammatory signalling pathways. Our results identified receptor membrane pore formation as essential but do not support packaging of TG2 within vesicles for release. P2X7R is integral to the NLRP3 inflammasome pathway that can also be triggered by caspase 4/5-mediated pyroptosis with associated gasdermin D pore formation. However, TG2 externalisation occurs in the absence of cell death (pyroptosis or apoptosis) and also inflammasome assembly, although its impact on the export process identifies partial mechanistic overlap. The conformational state of TG2 also critically affects export, identifying nucleotide-binding as essential.

Our results show that P2X7R triggered membrane pore activity directly correlates with TG2 export. Correspondingly, P2X7R polymorphisms affecting membrane pore formation also affect extracellular levels of proteins secreted via this pathway, impacting on TG2-mediated protein modifications and consequently the inflammatory response in immune-mediated diseases.

ABSTRACTS – POSTER PRESENTATIONS
TISSUE TRANSGLUTAMINASE CORRELATES WITH DISEASE PROGRESSION AND EPITHELIAL-MESENCHYMAL TRANSITION IN COLORECTAL CANCER CELLS
Ayinde O, Wang Z and Griffin M
School of Life & Health Sciences, Aston University, Birmingham, B4 7ET.

Background: Colorectal cancer is the third largest cancer globally and the metastatic disease presents a significantly poorer survival and clinical outcome. Epithelial to mesenchymal transition (EMT), acquisition of cancer stem like properties and drug resistance are cellular processes associated with tumour progression. Tissue transglutaminase (TG2) a multifunctional protein has been associated with these processes in certain cancer and fibrotic conditions. This study employs three well characterised human colon cancer cell lines (CRCs) RKO, SW480 and SW620 to investigate the involvement of TG2 and its therapeutic potential in tumour advancement and aggression.

Methods: TG2 expression was manipulated in the different CRCs by shRNA or TG2 transduction and the effect of TG2 small molecule inhibition was assessed on cellular markers of EMT, and tumour-sphere formation.

Results: TG2 expression was found to correlate with the advancement of the original tumour and with the presence of markers of EMT and drug resistance. TG2 was highly expressed in the metastatic cell line SW620 when compared to the primary cell lines SW480 and RKO and was the only cell line capable of forming tumour spheroids under non-adherent conditions. Manipulation of TG2 in the different CRCs by shRNA or TG2 transduction confirmed the relationship between TG2 and EMT. More so TG2 knockdown sensitised cells to 5 fluorouracil the conventional chemotherapeutic drug for CRCs, and the expression of EMT markers and TG2 were also observed to be upregulated in colorectal cancer cells with stem like properties. Interestingly application of TG2 site specific inhibitor attenuated EMT and the acquisition of stem like properties.

Conclusion: TG2 may play a role in tumour progression in human CRCs through its involvement in EMT and the acquisition of cancer stem cell like properties, and could hold both prognostic and therapeutic significance in colorectal cancer.
RNA ANALYSIS INDICATES TIGHT CORRELATION BETWEEN THE EXPRESSION OF A LONG NON CODING RNA AND THE TG2 mRNA LEVELS

Minotti L, Baldassari F, Volinia S, Bergamini CM and Bianchi N
University of Ferrara, Ferrara, Italy

The long non-coding RNAs (lncRNAs) are matter of intense investigation as potential regulators of gene expression, but their mechanism of action, activity and eventual therapeutic utility are not fully understood.

In the case of the transglutaminase 2 gene (TGM2) the databases of genome sequence indicate location of a lncRNA (LOC107987281) within the first intron. This lncRNA is 588 nt long, arises from 2 exons and starts few nucleotides (nt) 3’ of the first splicing site of TGM2 gene.

In this study we looked for correlations between expression of LOC107987281 lncRNA and TGM2 mRNA by real time PCR in K562 cell line untreated or treated with the anticancer drugs TPA (12-O-Tetradecanoylphorbol-13-acetate), Docetaxel and Doxorubicin. According to our data the drugs increase the transcript levels of both TGM2 mRNA and lncRNA.

To validate this finding we used HumanExon1_0ST Affymetrix; data were chip background-adjusted, quantile-normalized and summarized using RMA robust multiarray analysis implemented in the R package. The probesets recognize sequences inside each exon, near intronic splicing sites and others located in the untraslated regions of TGM2 gene. The analysis of total RNA samples from K562, HL60, THP-1 and U937 cell lines, untreated or treated with TPA in replicated experiments confirmed our earlier results. Results demonstrate correlation between LOC107987281 and TGM2 mRNA variant 1 in the cell lines (K562, HL60 and THP-1) where increased levels of TGM mRNA are produced.

Additional array study on 358 samples of several normal and tumor tissues leads to the same conclusions, indicating a correlation between TGM2 variant 1 mRNA and LOC107987281 lncRNA in relation to development of several tumors.
In recent years the use of collagen as a biomaterial has become very popular. Not only is it the most common type of protein found in the body, it also has low immunogenic properties. Tissue transglutaminase (TG2) is a member of a small family of enzymes with a reputation of being 'Nature's glue'. One of the abilities that is being investigated in this investigation is how transglutaminases are able to crosslink type I collagen via ε(γ-glutamyl) crosslinks. By cross-linking collagen in this way it increases the mechanical strength of collagen as well as making it more resistant to enzymatic degradation. Another much smaller non mammalian transglutaminase from *Streptomyces Mobaraensis*, is microbial Transglutaminase (mTG). Our previous work demonstrated that TG2 and mTG can crosslink collagen I to generate a biomaterial which is able to support osteoblasts differentiation and mineralization.

In this project, the effects of TG2 and mTG crosslinked collagen were compared to native collagen to assess differences in biocompatibility, degradation and its mineralisation by human osteoblasts (HOBs). It was found that both HOBs seeded onto TG2 and mTG treated collagen proliferated much more than on native collagen (where n=3 and p<0.05). Cell degradation of collagen was also found to be reduced on TG2 and mTG crosslinked collagen and the mineralisation rate was also when compared to native collagen (where n=3 and p<0.05). When investigation crucial integrin expression on cells it was found that crosslinked collagen was seen to cause an increased expression of β1, β3, α5 and αV integrins. These integrins are crucial to triggering the cell differentiation and mineralisation process in cells (where n=2). The addition of nanoparticle glass to the crosslinked collagen as means of increasing its mechanical strength was found not to alter the biocompatibility (where n=3).
THE INTERACTION BETWEEN TG2 AND SYNDECAN-4 IS CRITICAL FOR TG2 AND FIBRONECTIN MATRIX DEPOSITION IN LUNG FIBROSIS
Fell SA¹, Wang Z¹, Nanthakumar C² and Griffin M¹.

¹ School of Life and Health Sciences, Aston University, Birmingham
² Department of Fibrosis DPU, Respiratory TA, GlaxoSmithKline, Stevenage, UK

Patients with idiopathic pulmonary fibrosis (IPF) have a median survival rate of less than 3 years. The disease is characterised by excessive matrix deposition of proteins, which includes proteoglycans. Proteoglycans (e.g. Syndecan-4) have been shown to facilitate externalisation of the matrix crosslinking enzyme tissue transglutaminase (TG2), thus increasing extracellular cross-linking of collagens and fibronectin which contribute to IPF progression. This study identifies the importance of the interaction between TG2 and syndecan-4 in the context of IPF, along with characterisation of potentially therapeutic antibodies. Stimulation of NHLF cells with TGFβ1 led to an increased interaction between TG2 and syndecan-4 and also increased the matrix deposition of both TG2 and fibronectin. The application of a TG2-specific inhibitor, which alters the conformation of TG2, inhibited the TG2 to syndecan-4 interaction and the increased matrix deposition of TG2 and fibronectin. IgM antibodies targeting the heparin-binding site of TG2 prevented the interaction between TG2 and syndecan-4 in NIH3T3 (TG2) cells and reduced the deposition of TG2 and fibronectin into the matrix. The interaction between TG2 and syndecan-4 is increased in response to pro-fibrotic cytokines that drive the increased matrix deposition of TG2 and fibronectin. The application of TG2-specific inhibitors and heparin-binding site targeted antibodies demonstrate that preventing this interaction between TG2 and syndecan-4 can markedly reduce TG2 and fibronectin matrix deposition. These data suggest that targeting this interaction could serve as a therapeutic avenue in fibrotic conditions that are characterised by excessive matrix deposition of proteins, such as fibronectin.
High activity of tissue transglutaminase (TGase 2) in various tumors is associated with both their increased metastatic and invasive potential and their resistance towards chemotherapy and radiation. This renders TGase 2 an attractive target for the development of agents that are capable of targeting the tumor-associated TGase 2 for both imaging and therapeutic approaches [1]. Two different conformations of TGase 2 are known, with the closed one being the major intracellular form. It functions as a GTP-binding protein at low Ca\(^{2+}\) levels, whereas transition to the open conformation by Ca\(^{2+}\) enables its transamidase activity leading to protein-protein cross-linking [2].

Characterization of the GTP-binding activity of TGase 2 was done by means of a fluorescence anisotropy assay using the literature known BODIPY FL-GTP\(_\gamma\)S [3]. This compound is internally quenched by an intramolecular stacking of the BODIPY and the guanosine moieties. It is commonly used in fluorescence-based assays that make use of the unfolding and thereby dequenching of the fluorophore upon binding. [3] Such behavior, however, interferes with the analysis of fluorescence anisotropy as the measured data have to be corrected [4].

To optimize this assay method, we investigated a small series of newly developed GTP- and GDP-analogues labeled with fluorescein for TGase affinity and change in fluorescence upon protein binding. All compounds show a significantly smaller increase in fluorescence intensity compared to the BODIPY FL-labeled nucleotide and – in some cases – an up to ten-fold superior binding affinity towards TGase 2. The fluorescence anisotropy assay was then validated for inhibition studies with GTP and GTP\(_\gamma\)S, which show IC\(_{50}\) values (64 nM and 109 nM, respectively) that are in agreement with literature data [5,6].

Further investigations included titration of the GTP/TGase 2 interaction with CaCl\(_2\) and inhibition studies with GDP and ATP as well as compounds targeting the acyltransferase domain of TGase 2.

References:
DEVELOPMENT AND CHARACTERIZATION OF HUMAN MELANOMA CELL LINES AND XENOGRAFT MODELS EXHIBITING DIFFERENT LEVELS OF TRANSGLUTAMINASE 2
Hauser S1, Aepler J1,2, Pufe J1, Wodtke R1,2, Pietsch M3, Löser R1,2 and Pietzsch J1,2
1 Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Dresden, Germany, s.hauser@hzdr.de
2 Technische Universität Dresden, Department of Chemistry and Food Chemistry, Dresden, Germany
3 Center of Pharmacology, University of Cologne, Cologne, Germany

Tissue transglutaminase (TGase2) is involved in the progression of many different tumor entities, including malignant melanoma, via antiapoptotic processes and mechanisms supporting cellular survival, adhesion, and epithelial-mesenchymal transition [1]. Accordingly, it has been shown that TGase2 expression is higher in metastatic and chemoresistant tumors compared to primary tumors, underlining its role during tumor progression [2]. Therefore, TGase2 represents an interesting target for the development of selective inhibitors for theranostics of progressive malignant melanoma. In order to evaluate potent candidate compounds in vitro and in vivo, suitable transgenic melanoma cell lines and xenograft models with different TGase2 expression and activity were developed.

A375 and MeWo cells, two human malignant melanoma cell lines with high and very low TGase2 expression, respectively, were stably transfected with a lentiviral pHATtrick-mCherry vector (mCherry control cells) and a lentiviral pHATtrick-TGase2 vector (TGase2 cells). The resulting cell lines differed in their TGase2 expression and activity, as determined by Western Blotting and fluorescence anisotropy assay [3]. Transfection and overexpression of TGase2 did not influence cell proliferation behavior. 5×10^6 cells of each cell line were injected subcutaneously in athymic nude mice (NMRI-Foxn1nu) to form tumor xenografts that differed in their growth characteristics as well as in their TGase2 expression and activity. TGase2 activity in tumors was evaluated ex vivo by incorporation of fluorescently labeled cadaverine derivatives, which could be inhibited by a selective TGase2 inhibitor. These results indicate that the established tumor xenograft models provide the opportunity to evaluate potent candidate substances for diagnosis and therapy of melanoma on the one hand and to investigate pathophysiological processes associated with TGase2 in detail on the other.

References:
A VALIDATED ALL ATOM MODEL FOR THE COAGULATION FACTOR XIII A2B2 HETERO-TETRAMERIC COMPLEX.
Singh S1, Nazabal A2, Ivaskevicius V1, Oldenburg J1 and Biswas A1

1Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Bonn, Germany
2CovalX, Zürich, Switzerland

Background: The structure and interface chemistry of the FXIIIA2B2 heterotetramer complex is not known, and is critical for development of drug delivery systems and diagnostics in regard to FXIII.

Aims: To use cross-linking chemistry with MS analysis to determine inter and intra-subunit interface residues within the FXIIIA2B2 heterotetramer complex. Furthermore, to use these residues as guiding constraints to generate an accurate all-atom model of the complex. To determine putative antigenic and complex disrupting sites on both subunits based on the all atom model of the FXIIIA2B2 complex.

Methods: The FXIIIA2B2 complex purified from the plasma concentrate FibrogamminP was subjected to chemical cross-linking and High-Mass MALDI MS analysis to determine the interlink peptides of the complex. The residues identified were used as guiding constraints to model the FXIIIA2B2 complex. Based on the model, residues disrupting heterotetramer assembly were predicted based on pseudo-binding energies and validated in vitro.

Results: The High-Mass MALDI MS analysis allowed the detection of the intact cross-linked protein complex, its molecular weight and its stoichiometry. Post proteolysis more than 85% of the sequence of FXIIIA2B2 complex is covered by the peptide mass fingerprint. The model generated from the interface residues detected in the MS coverage is very stable as observed from a long MD simulation. Majority of the predicted-interface residues tested in vitro showed complex disrupting features post mutation. Similarly a number of previously reported mutations in both A and B subunits were mapped to regions which would disrupt the heterotetrameric complex. We could also identify key regions of electrostatic interaction between A and B subunits which can potentially be targeted by surface peptides for competitive inhibition.

Conclusion: We have been able to generate and validate an accurate all atom model of the FXIIIA2B2 heterotetramer based on experimental data.
INCREASED ACTIVITY OF HUMAN TRANSGLUTAMINASE 2 IN THE ERYTHROCYTES OF PATIENTS WITH SICLE CELL DISEASE
Sipka S
The National Institute of Arthritis, Metabolism and Digestive Diseases, NIH.
U.S. A.

Background: The role of human transglutaminase 2 in the Ca$^{2+}$ mediated crosslinking of erythrocyte proteins was observed earlier (Lóránd L. et al, 1976). Sickle Cell Disease (SCD) as a disease of pain and suffering that affected black Americans, became a political symbol for patients, doctors and politicians in the U.S.A. (Mc Williams JM et al. 2017).

Aim: During my research visit in 1978 in Kálmán Laki’s Lab (NIH, Bethesda) I started a work on the measurement of human transglutaminase 2 in the erythrocytes of patients with SCD. Now I am presenting those results what I could achieve there during my visit with a limited time but strongly related to Washington. Returning home I could not continue this work.

Methods: The 8 SCD patients (4-4 men and women) were collected from the Howard University Hospital (Washington, D.C.) specialized for black people. The healthy controls consisted of 2-2 black men/ women and 2-2 white men/ women with the ages of 20-40 years. The activity of transglutaminase was measured by the uptake of $^{14}$C putrescine by pooled and washed erythrocytes in Ca$^{2+}$ milieu. The proteins of ghost cell membranes were analyzed by PAGE.

Results: The formation of high molecular weight, non-disulfide bonded, $^{14}$C putrescine containing protein polymers (the products of transglutaminase) was increased in the membranes of sickle cells.

Conclusion: In the sickle cells a higher activity of human transglutaminase 2 was observed than in the healthy controls. This fact may explain the present good therapeutic effects of hydroxyurea in SCD (McWilliams JM et al 2017). However, the drugs of nitroso-urea like BCNU are inhibitors for human transglutaminase 2 (Laki K, Sipka S, 1978).
COVISOLINK™: NEW BACTERIAL TRANSGLUTAMINASE Q-TAG SUBSTRATE FOR THE DEVELOPMENT OF SITE SPECIFIQUE ANTIBODY DRUG CONJUGATES

Sivado E1-2, Thomas V1, El Alaoui M2, Dyson MR3, McCafferty J3 Valsesia-Wittmann S2 and El Alaoui S1

1Covalab, 11 Bd Einstein 69100 Villeurbanne, France
2Centre Léon Bérard, Innovations in Immunotherapy Platform, 28 rue Laennec 69008 LYON, France
3IONTAS Ltd, Babraham Research Campus, Babraham, Cambridge, CB22 3AT UK

CovIsoLink™ (Covalently Isopeptide crosslinking) relates to methods for enzymatic covalently coupling drugs and other compounds through transglutaminase site specific generated in the targeted proteins including, polypeptides, proteins and immunoglobulins (patent pending1). Transglutaminases (TGases) catalyze covalent cross-linking of specific glutamine residues to the primary amine of peptide-bound lysine residues or primary amines of other compounds such as polyamines2. Using an in house peptide library and the transglutaminase colorometric assay3, we identified several amino acid sequences that were recognized as glutamine donor substrates. The optimum peptide sequences were selected and we further confirmed that these sequences have improved affinity compared with the conventional small peptides Z-QG. In different experiments we engineered Fc-containing polypeptide at the C-terminal domain and showed that mTG incorporates with high efficiency several types of amine donors to the engineered antibodies. CovIsoLink™ is now used to develop new antibody drug conjugates (ADCs) since the major advantage of this method is to obtain a homogenous immunoconjugate with uniform stoichiometry.

We developed and characterized different recombinant anti Her2 IgG1 Mab carrying optimized enzymatic substrates (tag) by genetic insertion in the coding sequence of MAb. We then evaluated the best linkers and conformation to incorporate different compounds through bacterial Transglutaminase (mTG) enzymatic reaction. We set up experimental conditions, production, purification, HPLC/MS analysis and control of the immunoreactivity of CovIsoLink™ Her2-ADC. Using mTG, we obtained site specific conjugation of different modified drugs with optimized linker on the anti Her2 IgG1 antibody. By HIC analysis, we validated a specific and reproducible DAR reaching DAR2 depending on drugs and experimental conditions. In vitro and In vivo characterization and dose response studies of CovIsolink-ADC specificity and reactivity are currently performed in Her2 positive models by comparison with Kadcyla (T-DM1).

TRANSGLUTAMINASE ACTIVITY REGULATES DIFFERENTIATION, MIGRATION AND FUSION OF OSTEOCLASTS VIA AFFECTING ACTIN DYNAMICS AND RHOA ACTIVITY

Sun H¹ and Kaartinen MT¹,²
¹ Faculty of Dentistry, McGill University, Montreal, QC, Canada
² Faculty of Medicine, Division of Experimental Medicine, McGill University, Montreal, QC, Canada

Osteoclasts are multinucleated macrophage lineage cells capable of resorbing mineralized bone. Increased osteoclast activity causes bone loss, i.e., osteopenia. Transglutaminases (TG) are a family of structurally and functionally related enzymes that catalyze the Ca²⁺-dependent crosslinking of proteins through introducing an isopeptide bond between a lysine and a glutamine residue. TG family is comprised of TG1-TG7 and Factor XIII-A (FXIII-A). TG2 and FXIII-A are both expressed in monocyte/macrophage lineage cells; however, their expression in osteoclasts and their potential role during osteoclastogenesis and osteoclast resorption have not so far been explored. To address the role of TGs in osteoclasts, we used murine bone marrow-derived macrophages (BMMs) which were differentiated into osteoclasts with M-CSF and RANKL. We report here that both macrophages and osteoclasts express mRNA of TG1, TG2 and FXIII-A. Immunofluorescence microscopic analysis showed all the three enzymes co-localized with podosomes in osteoclasts. To examine the role of TG activity in osteoclastogenesis, BMMs were treated during the osteoclastogenesis with NC9 - an irreversible TG inhibitor. Osteoclast size was decreased dramatically with low concentrations of NC9 and osteoclast differentiation was blocked completely with higher concentrations. When NC9 was added to the osteoclast precursors at different stages, it inhibited the differentiation, migration and fusion of pre-osteoclasts. Consistently, resorption pit assay showed that osteoclast resorption activity was inhibited by NC9 treatment. In addition, osteoclast podosome belt formation was found to decrease when treated with NC9 suggesting that TG activity regulated actin dynamics in osteoclast precursors. Finally, the levels of RhoA, regulator of actin dynamics and podosome belt/sealing zone formation, was found significantly elevated in NC9 group compared to control group and the inhibitory effect of NC9 on osteoclastogenesis was reversed by RhoA inhibitor. Taken together, our data suggests that TG activity regulates osteoclastogenesis via affecting cytoskeletal and actin dynamics and RhoA activity.
CHARACTERISATION OF THE ISOPEPTIDASE ACTIVITY OF HUMAN BLOOD COAGULATION FACTOR XIII-A

Szabó Z, Fésüs L and Király R

Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Human coagulation factor XIII-A (FXIII-A) is a member of the transglutaminase enzyme family. Physiologically after thrombin activation it catalyses the formation of γ-glutamyl-ε-lysine crosslinks within fibrin clots. This enzyme has several other intra- and extra-cellular functions. Interestingly, transglutaminases could cleave the previously formed isopeptide bond which activity is poorly studied. Previous works demonstrated that FXIII-A is able to cleave out α2-plasmin inhibitor from the fibrin clot potentially regulating both pro- and anticoagulation processes (Mimuro et al. 1986. J Clin Invest).

In this study our goal was to characterise the isopeptidase activity of FXIII-A on protein and peptide based substrates.

In our laboratory, a recently developed protein-peptide based TG2 isopeptidase assay was successfully adopted for commercially available human FXIII-A (expressed in insect cells, Zedira) replacing the TG2 specific FLpepT26 peptide for α2-plasmin inhibitor derived FLpepPI2 peptide which earlier was found as a good substrate for FXIII-A transamidase activity.

Then, the isopeptidase activity of FXIII-A was tested on cross-linked fibrin degradation products using a recently developed commercial monoclonal antibody (Zedira) which directly recognises the crosslinked fibrin neoeptope. Applying various conditions we have not been able to observe cleavage of cross-linked D-D fibrin dimer either on Coomassie Brilliant Blue stained SDS-polyacrylamide gel or by Western blot.

Based on our results we intend to develop further methods to study and later separate or modify isopeptidase activity of FXIII-A for potential therapeutic use.

This work was supported by Research University grant from the University of Debrecen (RH/885/2013), OTKA NK 105046.
Albeit the significant advances in the chemotherapy, cancer has continued to increase. It is becoming obvious that the long-term survival is mainly dependent on the early diagnosis and treatment of the disease yet there is no absolute treatment that is 100% effective against disseminated cancer such as metastatic renal cell carcinoma (mRCC). mRCC makes up to 3% of all malignant tumors in adults and has first-line treatment with multikinase inhibitor Sorafenib followed by mTOR inhibitor Everolimus. Tissue transglutaminase (TG2) was reported to be involved in the drug resistance as the increased expression of TG2 in RCC results in tumor metastasis with a significant decrease in disease- and cancer-specific survival outcome. The aim of this study is to understand the role of transamidating and GTP-binding activity of TG2 in response mechanism of mouse epithelial kidney cancer cells, RenCa to Sorafenib and Everolimus. For this purpose, RenCa cells were transduced with lentiviral particles encoding wtTG2, transaminase-null TG2-C277S form with low GTP-binding affinity, GTP-binding deficient form TG2-R580A, and transaminase-inactive TG2-W241A. In order to evaluate the drug-resistance phenotype of TG2 mutants, cell proliferation assay (WST-1) and Annexin-V staining was performed following the treatment with Sorafenib and Everolimus in time- and dose- dependent manner. Our results showed that non-transduced control, wt-TG2, TG2-C277S and TG2-R580A mutant RenCa cells demonstrated a similar cell viability pattern with a 30% and 15% cell survival against Everolimus and Sorafenib, respectively. On the other hand, 2- and 3- fold increase in the cell survival was detected for the TG2-W241A cells after Sorafenib and Everolimus treatment. Our data supports the notion that GTP-binding activity of TG2 is closely related with the development of drug-resistance phenotype in RenCa cells, suggesting that inhibitors targeting the GTP-binding activity of TG2 may serve long-lasting advantage of anti-cancer treatments in RCC.
THE FUNCTIONAL RELATIONSHIP BETWEEN TG2 AND TGFβ1 AND ITS IMPORTANCE AS A THERAPEUTIC TARGET IN HUMAN DISEASE

Griffin M and Wang Z

School Life and Health Sciences, Aston University, Aston Triangle, Birmingham, UK
TRANSGLUTAMINASE TYPE 2 INTERACTS WITH GRP75 AND INFLUENCES THE Ca\(^{2+}\) HOMEOSTASIS AT THE ER/MITOCOCHONDRIA (MAM) CONTACT SITES

D'Eletto M\(^1\), Rossin F\(^1\), Facenda D\(^2\), Campanella M\(^2\) and Piacentini M\(^3\).

\(^1\)Department of Biology, University of Rome ‘Tor Vergata’, Rome, 00133, Italy
\(^2\)Department of Biology, University of Rome ‘Tor Vergata’, Rome, 00133, Italy; Department of Comparative Biomedical Sciences, The Royal Veterinary College London and UCL Consortium for Mitochondrial Research, Royal College Street, NW1 0TU London, UK
\(^3\)Department of Biology, University of Rome ‘Tor Vergata’, Rome, 00133, Italy; National Institute for Infectious Diseases, IRCCS “Lazzaro Spallanzani”, Rome, Italy

Transglutaminase type 2 (TG2) is a multifunctional enzyme with different subcellular localizations. In the presence of high calcium levels, TG2 catalyzes a vast array of protein post-translational modifications, including protein–protein crosslinking, incorporation of primary amines into proteins. Recently, in order to identify the TG2’s binding partners, we have carried out the enzyme interactome analysis by utilizing Tandem Affinity Purification approach combined with HPLC and MALDI TOF/TOF mass spectrometry. Our interactome analysis has revealed that TG2 interacts with various proteins belonging to different functional categories such as chaperones, mitochondria, metabolism and cytoskeleton. Among these, particularly interesting is the GRP75 (glucose-regulated protein75), a molecular chaperone belonging to the heat shock protein 70 family. GRP75 is involved in facilitating the transport of Ca\(^{2+}\) from the endoplasmic reticulum to mitochondria, acting as a bridge between the two organelles and assembling the complex IP3R-GRP75-VDAC. These complexes are localized in the mitochondria-associated membranes (MAM). The MAM constitute the site of contact between the ER and mitochondria, providing Ca\(^{2+}\)-rich microdomains necessary for mitochondrial signaling.

We confirmed the interaction between TG2 and GRP75 and we demonstrated that the interaction between the two proteins occurs primarily in the mitochondrial fraction. Interestingly, we show the presence of TG2 in the MAM region. Interestingly, the presence of TG2 influences the interaction between IP3R and GRP75, resulting in an impaired ER-mitochondrial Ca\(^{2+}\) flux.
ATTACHMENT OF SMALL PROTEIN MODIFIERS BY TRANSGLUTAMINASES
Demeny MA and Fesus L
Stem cell, Apoptosis and Genomics Research Group of the Hungarian Academy of Sciences, University of Debrecen, Hungary

The recognition of the significance of protein modification by covalent conjugation to other proteins has deepened immensely in the last decade thanks to the discovery of a bevy of ubiquitin-like (UBL) proteins and their roles on a spectrum from transcription to immune signaling. In line with simpler posttranslational attachments the presence of protein appendages is translated into functional outcome through recognition by docking proteins, but unlike smaller phosphate, methyl, etc. groups this binding does not involve motifs around the site of attachment, but is limited to properly configured surfaces patches of the attached proteins themselves.

Beyond the ubiquitousness of UBL systems covalent protein-protein conjugation systems are rare in biology. Although different in mechanistic detail, in some aspects transglutaminase (TG) catalyzed protein cross-linking is analogous to primitive Ub-like systems. The TransDab database counts Ub among TG substrates and our previous analysis of heavily ubiquitylated inclusions from Alzheimer’s disease patients uncovered N-γ-(glutamyl)-ε-lysine bonds between Ub, α-synuclein and Hsp27. TG mediated protein cross-linking is ATP-independent but calcium-dependent, consequently it is likely enhanced in stressed cells. The analogy of the two processes and the laxity of the rationale of recognition for the type of the actual attachment bond of UBLs prompted us to test if under particular conditions TGs can also mediate attachment of small protein modifiers with a functional consequence.

Here we report on testing the ability of purified recombinant members of the TG family to append various UBL proteins to putative substrates, including the ones identified in neurodegenerative disease, in in vitro reconstituted reactions.
Transglutaminase 2 (TG2) is a multifunctional and ubiquitous protein that can contribute to the development of various diseases such as celiac disease (CD). Structurally, TG2 is composed of four distinct domains: an N-terminal β-sandwich, a catalytic core and two C-terminal β-barrel domains. Catalysis of Ca$^{2+}$-dependent posttranslational modification of proteins is the major hallmark of TG2. It is well-known that in the presence of calcium there is a significant interdomain interaction between the catalytic core domain and domains 3 and 4, which increases the accessibility of the active centre for the transamidase activity. The question is whether the N-terminal domain itself or the N-terminal and core domains’ interplay are implicated in the TG2 functions. To answer this we started to manipulate mouse TG2 enzyme. In spite of the fact that the sequence homology of mouse and human TG2 enzymes is about 86%, mouse TG2 has much higher Ca$^{2+}$-sensitivity and therefore works with higher activity at lower Ca$^{2+}$ concentrations. In our study we compared structural features of the core domain and the N-terminal domain by homology modelling and site-directed mutagenesis. Structural analysis revealed subtle differences in the interface of domains and in the Ca$^{2+}$ binding sites. A point mutation of one positively charged amino acid side chain related to the S4 Ca$^{2+}$ binding site in the mouse TG2, into a neutral side chain led to an increase in protein cross-linking activity, which can potentially further increase the affinity for Ca$^{2+}$. Also, changes in the N-terminal domain influenced cross-linking activity, probably by influencing the conformation of the protein. This work was supported by GINOP-2.3.2-15-2016-00015 and NKFI 120392. *MN’s participation in this study was covered by Erasmus Student Mobility Program between University of Surrey and University of Debrecen.
Transglutaminase 2 (TG2) mediated stabilization of protein assemblies has a pivotal function in tissue repair. However, aberrant TG2 activity has been linked to fibrosis and autoimmunity. We have previously demonstrated that TG2 export is linked to activation of purinergic receptor P2X7R, and that through co-secretion of thioredoxin-1, this mechanism controls extracellular levels of active enzyme.

**Objectives**

Here, we investigate the relationship between inflammasome assembly and TG2 secretion in innate immune cells, as well as a potential role of thioredoxin-1 activity in TG2 secretion.

**Experimental approach**

Cell models included macrophages derived from human peripheral blood monocytes, THP-1 cells and P2X7R-expressing HEK293 cells. TG2 and thioredoxin-1 secretion was assessed by Western blotting.

**Results**

Gasdermin D activation by caspase-4/5 triggers pyroptosis and is part of the NLRP3, AIM2 and NAIP-NLRC4 inflammasome pathways. Stimulation of P2X7R activation alone or alongside gasdermin D activation in human macrophages, demonstrated that TG2 externalisation occurs independently of pore formation by cleaved gasdermin D, does not require inflammasome assembly, and occurs in the absence of cell death, instead relating to P2X7R membrane pore formation. TG2 secreted by macrophages in response to P2X7R activation is a ~66kDa truncated form. We show that this is generated in a processing event linked to NLRP3 inflammasome assembly and hence, this processing does not occur in HEK293-P2X7R cells. We further demonstrate that TG2 secretion does not require thioredoxin-1 activity.

**Discussion**

This work demonstrates that TG2 secretion on P2X7R activation is not related to cell death but is linked to P2X7R membrane pore formation. Polymorphisms affecting this function of P2X7R may therefore affect the extracellular levels of TG2, which may impact TG2-mediated protein modifications, potentially affecting the inflammatory response in pathology.
THE DYNAMIC NATURE OF TRANSGLUTAMINASES
Hjorth-Jensen S\textsuperscript{1}, Richard D\textsuperscript{1}, Stephenson S\textsuperscript{1}, Walsh T\textsuperscript{1} and Croll, T\textsuperscript{2}
\textsuperscript{1}Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia
\textsuperscript{2}Cambridge Institute for Medical Research, University of Cambridge, Cambridge, CB2 0XY, UK

Introduction: Transglutaminases incorporate various primary amines into specific glutamine residues of target proteins via a transamidation reaction. N\textsubscript{ε}(γ-glutamyl)lysine crosslinks result should a lysine residue be incorporated, or alternately, biogenic amines may be incorporated to form protein-amine conjugates. While it has been suggested that transamidation may be reversible, this has never been demonstrated and it is assumed (aside from hydrolysis of products) transglutaminase activity is unidirectional. This project demonstrates transamidation is reversible and proceeds towards a dynamic equilibrium involving constant interconversion between each possible transamidated product. This implies, in a biological context, fluctuations in amine concentrations effect conversions between transamidated product varieties.

Methodology: Investigations utilised a fluorophore-labelled glutamine substrate, a quencher-labelled lysine substrate, and their fluorescently-quenched N\textsubscript{ε}(γ-glutamyl)lysine product. Transglutaminase reactions were run in the forward (from free substrates) and reverse (from product) direction, under varying concentrations and in the presence of varying amounts of competitive amines.

Results: Transglutaminase activity was shown to be freely reversible; under equivalent conditions the forward and reverse reactions converged to similar FIs at equilibrium. Furthermore, when the quenched product was incubated in the presence of histamine, the incorporated lysine substrate was exchanged with the unlabelled histamine.

Conclusions & Significance: Crosslinks may be reversibly formed or broken by transglutaminase activity as incorporated lysines are interchanged with small amines. Transglutaminases may therefore play a more dynamic role in biology than currently believed, with reversibility key to understanding many of their biological roles. This behaviour may explain why TG2-mediated serotonin incorporation is necessary for its induction of vascular smooth muscle contraction. Following an influx of serotonin, transglutaminase activity would serve to reversibly exchange stabilising N\textsubscript{ε}(γ-glutamyl)lysine crosslinks for monoamine adducts within structural/contractile proteins, resulting in destabilisation facilitative of contraction.

In conclusion, the insight provided by this project will lead to a greater understanding of transglutaminase biology and potentially lead to new avenues for disrupting disease processes.
LIPOMA-PREFERRED PARTNER PROTEIN (LPP), A GENETIC MARKER FOR COELIAC DISEASE PREDISPOSITION IS INTERACTING WITH TRANSGLUTAMINASE 2 (TG2)

Kerekesné Tóth B¹, Bogáti R¹, Kerekes T¹, Hegymeginé Elek R¹, Király R¹, Simon-Vecsei Zs¹, Caja S², Bartáné Tóth B¹, Nadalutti C², Mäki M², Fészus L¹ and Korponay-Szabó IR³

¹ Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary
² Coeliac Disease Study Group Tampere Center for Child Health Research, University of Tampere, Tampere, Finland
³ Department of Paediatrics, University of Debrecen and Heim Pál Children's Hospital, Debrecen and Budapest, Hungary

Objectives and Study: Coeliac disease (CD) is an intestinal disorder triggered by dietary gluten from wheat, rye and barley and is characterized by small-intestinal lesion with villous atrophy, crypt hyperplasia and generation of autoantibodies against TG2. It is a permanent gluten intolerance segregating in families and is inheritable. The genetic risk for coeliac disease is determined by the presence of HLA-DQ2 or DQ8 and other non-HLA genes with lower contribution. The LPP gene encoding a structural protein important for cell motility shows high association with CD in four consecutive intronic single nucleotide polymorphisms. It is currently unknown how LPP in involved in the disease pathogenesis. The aim of this study was to investigate whether LPP can associate with TG2 or targeted by serum antibodies from coeliac disease patients.

Methods: Biopsy samples from 6 normal and 5 coeliac disease patients as well as human umbilical cord endothelial vein (HUVEC) cells derived from normal and coeliac subjects were analysed for LPP expression by real time PCR and immunohistochemistry. Human recombinant LPP and TG2 (full length and various domain/point-mutants) were expressed in E. coli and used for interaction studies by enzyme-linked immunoassay (ELISA) and surface plasmon resonance real-time binding analysis (Biacore).

Results: LPP was similarly expressed in normal and coeliac tissues. Both TG2 and LPP localised to focal adhesion complexes and associated with the cytoskeleton of muscle cells maintained in culture. Recombinant LPP bound dose-dependently to TG2 and the interaction involved multiple domains of TG2. Serum IgA antibodies from coeliac disease patients showed a higher reaction with recombinant LPP in ELISA than those from non-coeliac control.

Conclusion: LPP is directly binding to TG2, the main coeliac autoantigen.
Transglutaminase 2 (TG2) is a multifunctional protein. Based on the analysis of exome databases human TG2 protein is well conserved suggesting its essentiality for humans, but TG2 KO mouse is viable and does not have disease manifestation in spite of the high sequence homology. Recently, our in silico study (Thangaraju et al. 2016. Amino Acids) revealed novel amino acid clusters in primates and human TG2 compared to the mouse enzyme, raising the possibility of gain of function during evolution.

We believe that systematic comparative study of human and mouse enzymes could reveal evolutionary gained new function. In this study our goal was the biochemical and functional comparison of human and mouse TG2.

First, both enzymes were expressed and purified from the same efficient bacterial system with similar yield and purity. In case of various substrates human TG2 presented lower transamidase activity and Ca$^{2+}$ sensitivity than mouse TG2. This correlates with the predictions showing the stabilising effect of specific amino acid clusters in the human enzyme. The human TG2 had also lower isopeptidase activity on peptide substrate than mouse TG2 but their isopeptidase activities on protein-peptide substrates were similar. There were no significant difference in their BODIPY-GTPγS binding although the human enzyme was more sensitive for GTPγS inhibition. Both exogenously added enzymes enhanced the adhesion of HEK AD cells to fibronectin coated surface. Identification of potential species specific TG2 substrates are in progress.

Based on our first set of results we may conclude that evolutional changes has decreased the calcium sensitivity of human TG2 compared to the mouse enzyme. Substrate specificity and the scaffolding function of TG2 could be also changed which are the targets of our future studies.

This work was supported by Research University grant from the University of Debrecen (RH/885/2013), OTKA NK 105046.
Recently, it has been discovered that cold exposure or adrenergic stimuli can provoke the appearance of clusters of UCP1-positive heat producing cells in white adipose tissue (WAT) through the process of browning. These inducible cells have been named ‘beige’ adipocytes and have an overlapping but distinct gene expression pattern compared to classical brown adipocytes. Previously, we found that TG2+/− mice could not tolerate acute cold exposure as TG2−/− animals. Both TG2−/− and TG2+/+ mice used up half of the triacylglycerol content of inguinal WAT (IWAT) after 3h treatment; however, TG2−/− mice still possessed markedly whiter and higher amount of epididymal WAT (EWAT) as reflected in the larger size of adipocytes and lower free fatty acid levels in serum.

In order to investigate the underlying molecular mechanism, we isolated preadipocytes from EWAT of animals and differentiated them to ‘beige’ direction. The differentiation process was successful as adipocytes with multilocular lipid droplets appeared with significantly increased expression of ‘beige’ marker genes UCP1, TBX1, TNFRFS9 and TMEM26 compared to preadipocytes. However, similarly to our previous results on whole EWAT samples, we did not detect any differences in TG2−/− cells compared to TG2+/+ cells. These results together indicate that the differentiation process is probably unaffected by TG2, and TG2−/− EWAT contains similar number of ‘beige’ cells compared to TG2+/+. Next, we injected the mice intraperitoneally with 10µg/g body weight arterenol 48 and 24 hours before the isolation of EWAT. We found that the tissue of TG2−/− mice remained again lighter compared to TG2+/+ suggesting a difference in the activation process of existing ‘beige’ cells. Hence, we currently investigate the characteristics of differentiated TG2−/− and TG2+/+ ‘beige’ cells after 4h adrenergic stimuli. Our results suggest that transglutaminase related regulatory processes will be possible targets for pharmacological interventions in obesity and metabolic disorders in the future.
ANALYSIS OF THE PROTEIN CROSSLINK-PROFILE CHANGES AND THE NEUTROPHIL EXTRACELLULAR TRAP PATTERNS ELICITED BY DIFFERENT STIMULI
Márkus B1,2,3, Kristóf E3, Csomós I4, Fésüs L3 and Csősz É1,2,3

1Biomarker Research group, 2Proteomic Core Facility, 3Department of Biochemistry and Molecular Biology, 4Department of Biophysics and Cell Biology, University of Debrecen

Mature neutrophils act as a first line of defence migrating to the site of infection in response to microbial invasion. They are able to attack pathogen directly in three different ways: phagocytosis, release of antimicrobial peptides and formation of neutrophil extracellular traps (NETs). NET is the result of a unique form of cell death in which neutrophils eject their mixture of nucleoplasm and cytoplasm into the extracellular space forming a web-like structure. Hereby the invaded pathogens are trapped, neutralized therefore their dissemination is inhibited. Several antimicrobial proteins and proteases (such as neutrophil elastase, myeloperoxidase, cathepsin G, azurocidin, lactoferrin) are essential elements of the NET contributing to direct and indirect antimicrobial activities. Based on MS/MS data of protein cross-links formed in the differently treated NETs we could observe protein cross-linking by chlorinated polyamines and transglutaminases and we could demonstrate that both myeloperoxidase and TG1 are required for the stabilization of NET (Csomos et al. Cell Death Dis. 2016). Various bacteria, fungi and viruses can induce NET formation therefore we aimed to investigate the changes in the protein crosslink-profile and in the complex web-like structure during NET formation elicited by different pathogens.
STRUCTURE-ACTIVITY RELATIONSHIPS OF POTENT, TARGETED COVALENT INHIBITORS THAT ABOLISH BOTH THE TRANSAMIDATION AND GTP BINDING ACTIVITIES OF HUMAN TISSUE TRANSGLUTAMINASE

McNeil NMR, Akbar A, Albert MR and Keillor JW*

Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa Ontario, Canada.

Human tissue transglutaminase (hTG2) is a multifunctional enzyme that is ubiquitously expressed in tissues. It is primarily known for its calcium-dependent transamidation activity that leads to formation of an isopeptide bond between glutamine and lysine residues; however, hTG2 is also a GTP binding protein. This GTP binding function distinguishes it from other members of the transglutaminase family and recently, elevated hTG2 expression and GTP-binding activity has been linked to cancer stem cell (CSC) survival and metastatic phenotype in certain cancer cells. A powerful anticancer strategy would involve the development of chemotherapeutics that target CSCs and through collaborative efforts, we have identified hTG2 as a potential therapeutic target. This work focuses on the development of potential therapeutic agents that selectively inactivate human hTG2. We have prepared a series of targeted covalent inhibitors (TCIs) based on our previously reported Cbz-Lys scaffold. From this structure-activity relationship (SAR) study, novel irreversible inhibitors were identified that block the transamidation activity of hTG2 and allosterically abolish its GTP binding ability, with a high degree of selectivity and efficiency. We will present and discuss our latest results in these directions.
Transglutaminases are a family of enzymes able to covalently crosslink a broad range of substrate proteins with available glutamine and lysine residues resulting in protease-resistant isopeptide bonds between the substrate residues. Moreover, transglutaminases can exert their functions also by interacting with various interaction partners. TRANSDAB (www.genomics.dote/wiki), an interactive database contains more than 500 transglutaminase substrate proteins and interaction partners. This database was generated to provide user-friendly information regarding substrate name, the type of the determination (in situ/in vitro), the source organism, subcellular localization and Uniprot ID of the protein and structural information, if available. The position of reactive glutamines and lysines, and of interacting residues are indicated. Currently, TRANSDAB contains 14 substrates for TG1, 173 substrates for TG2, 16 substrates for TG3, 3 substrates for TG4, 4 substrates for TG5, 10 substrates for microbial TG and 133 substrates for FXIIIa. Among the TG2 substrates there are 56 glutamine donor, 29 lysine donor and 88 glutamine and lysine donor substrates. The residues serving as substrates for TG2 are known in case of 34 glutamine substrates, 13 lysine substrates and 30 proteins where both the glutamine and lysine residues serve as substrates for the enzyme. TRANSDAB contains 116 glutamine donor, 3 lysine donor and 14 glutamine and lysine donor substrates for FXIIIa, and the position of substrate residues were determined in case of 114 glutamine, 3 lysine and 5 glutamine and lysine donor substrates. Additionally TRANSDAB contains 6 substrates for kinase and 2 for deamidase activities of TG2. Beside the substrates, 80 interaction partners of TG2 are included. Our aim has been to update this database by collecting the transglutaminase substrate proteins and interaction partners which have been published in the scientific literature by creating an easily accessible and user friendly website which can be edited by the scientific community.
Type 2 transglutaminase (TG2) is a calcium-dependent cross-linking enzyme known to be implicated in multiple diseases linked to calcium dysregulation, e.g. oxidative-stress, cell-death and neurodegeneration. Here we investigated TG2 activity in rat hippocampal neurons and its impact on neuronal calcium homeostasis. Neurons treatment with tetrodotoxin (TTX), an inhibitor of synaptic transmission, led to a 2.4-fold increase in TG2 transamidating activity. To verify if this effect could represent a compensatory response aimed at rescuing neuronal firing we analysed how manipulation of TG2 activity impacted synchronous calcium oscillations, a form of intense synaptic activity driven by bursts of neuronal firing. TG2 inhibition decreased calcium oscillations’ frequency and the interspike calcium concentrations in fura-2-loaded neurons, suggesting a role for TG2 in sustained excitatory transmission. Conversely, TG2 promoted the onset of calcium transients followed by sustained plateaus and reversible block of calcium oscillations. Overexpression of human TG2 by transient transfection induced a significant rise in basal calcium concentration, evaluated in the presence of TTX. Basal calcium increase mediated by TG2 did not occur in calcium-free medium, revealing that the protein induces influx of extracellular calcium ions. Nimodipine, a blocker of L-type voltage-gated calcium channels (VOOCs), caused a partial recovery of calcium concentration towards resting levels when applied during the TG2-induced plateau phase, suggesting that calcium influx partially occurs through L-type VOCCs. Consistently, VOCCs-mediated calcium influx was enhanced by TG2. However, other channels/transporters may contribute to this process. Few putative TG2 targets identified by a proteomic approach are now under investigation. Collectively these data indicate a possible role of TG2 in the enhancement of basal calcium levels and in the facilitation of calcium transmission through VOCCs in neurons.
UNCONVENTIONAL EXPORT OF TRANSGLUTAMINASE 2: ROLE OF CO-FACTORS REGULATING ENZYME ACTIVITY AND CONFORMATIONAL STATE.
Turberville S, Griffiths R, Beck K, Aeschlimann P, and Aeschlimann D

Matrix Biology and Tissue Repair Research Unit, School of Dentistry, and Arthritis Research UK Biomechanics and Bioengineering Centre of Excellence, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK

Cells secrete transglutaminase-2 (TG2) via an unconventional protein export mechanism. Our group has shown that purinergic signalling controls TG2 export, and has implicated P2X7 receptor activation. Intra- and extracellular TG2 functions depend on mutually exclusive conformations induced by GTP and Ca\textsuperscript{2+} binding, respectively. This allosteric regulation enables an extended conformation with transamidase activity extracellularly, driven by Ca\textsuperscript{2+}-binding, and a compact conformation stabilized by nucleotide binding intracellularly. Here, we investigate how mutations affecting the conformational state of TG2 affect its externalisation, with a focus on the nucleotide-binding site.

Objective
We aim to assess whether conformational changes upon GTP binding or G-protein functionality of TG2 are part of the mechanism enabling externalisation.

Methods
TG2 mutant cDNAs were generated by site-directed mutagenesis. Secretion of TG2 mutants in response to P2X7R activation was assessed in HEK293-P2X7R cells following cDNA transfection. TG2 mutant proteins were generated in E.coli for biochemical studies investigating GTP binding, GTPase activity, and transamidase activity. Structural analysis was performed using circular dichorism spectroscopy (CD).

Results
Cells failed to secrete TG2\textsuperscript{K173N/F174D} and R\textsuperscript{580A} following stimulation with P2X7R agonist, and secretion of TG2\textsuperscript{K173L} but not TG2\textsuperscript{K173N} was impaired in comparison to wild-type TG2 (wtTG2). CD revealed no overt structural changes between wtTG2 and the GTP binding site mutants. Mutants with impaired export displayed reduced binding affinity for GTP as detected in direct binding assay as well as by inhibition of isopeptidase activity using GTP, with a correlation between GTP binding affinity and protein export. No GTP binding was observed for TG2\textsuperscript{K173N/F174D} and R\textsuperscript{580A}. Similarly, wtTG2, TG2\textsuperscript{K173N} and TG2\textsuperscript{K173L} displayed GTPase activity, whilst no activity was measurable for TG2\textsuperscript{K173N/F174D} and R\textsuperscript{580A}.

Conclusion
The work presented here demonstrates that the ability of TG2 to bind GTP is essential to its active export from cells via the pathway regulated by purinergic signalling.
LIST OF PARTICIPANTS
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daniel Aeschlimann</td>
<td>Cardiff University</td>
<td><a href="mailto:AeschlimannDP@Cardiff.ac.uk">AeschlimannDP@Cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Robert Ariëns</td>
<td>University of Leeds</td>
<td><a href="mailto:r.a.s.ariens@leeds.ac.uk">r.a.s.ariens@leeds.ac.uk</a></td>
</tr>
<tr>
<td>Oluseyi Cyril Ayinde</td>
<td>Aston University</td>
<td><a href="mailto:ayindeoc@aston.ac.uk">ayindeoc@aston.ac.uk</a></td>
</tr>
<tr>
<td>Zoltán Balajthy</td>
<td>University of Debrecen</td>
<td><a href="mailto:balajthy@med.unideb.hu">balajthy@med.unideb.hu</a></td>
</tr>
<tr>
<td>Barbara Baráth</td>
<td>University of Debrecen</td>
<td><a href="mailto:barath.barb11@gmail.com">barath.barb11@gmail.com</a></td>
</tr>
<tr>
<td>Beáta Barta-Tóth</td>
<td>University of Debrecen</td>
<td><a href="mailto:toth.beata@med.unideb.hu">toth.beata@med.unideb.hu</a></td>
</tr>
<tr>
<td>Charlotte Anneke Bäuml</td>
<td>University of Bonn</td>
<td><a href="mailto:charlotte.baeuml@uni-bonn.de">charlotte.baeuml@uni-bonn.de</a></td>
</tr>
<tr>
<td>Carlo Bergamini</td>
<td>Universita' di Ferrara</td>
<td><a href="mailto:cfr@unife.it">cfr@unife.it</a></td>
</tr>
<tr>
<td>Nicoletta Bianchi</td>
<td>Universita' di Ferrara</td>
<td><a href="mailto:ivano.pampolini@unife.it">ivano.pampolini@unife.it</a></td>
</tr>
<tr>
<td>Arijit Biswas</td>
<td>University Clinic Bonn</td>
<td><a href="mailto:Arijit.Biswas@ukbonn.de">Arijit.Biswas@ukbonn.de</a></td>
</tr>
<tr>
<td>Réka Bogáti</td>
<td>University of Debrecen</td>
<td><a href="mailto:reka.bogati@outlook.com">reka.bogati@outlook.com</a></td>
</tr>
<tr>
<td>Anna Bychkova</td>
<td>N.M. Emanuel</td>
<td><a href="mailto:anna.v.bychkova@gmail.com">anna.v.bychkova@gmail.com</a></td>
</tr>
<tr>
<td>Haris Choudhery</td>
<td>Aston University</td>
<td><a href="mailto:choudheh@aston.ac.uk">choudheh@aston.ac.uk</a></td>
</tr>
<tr>
<td>Eva Csosz</td>
<td>University of Debrecen</td>
<td><a href="mailto:cseva@med.unideb.hu">cseva@med.unideb.hu</a></td>
</tr>
<tr>
<td>Manuela D'Eletto</td>
<td>University of Rome</td>
<td><a href="mailto:manuela.deletto@gmail.com">manuela.deletto@gmail.com</a></td>
</tr>
<tr>
<td>Mate Agoston Demeny</td>
<td>University of Debrecen</td>
<td><a href="mailto:demenym@med.unideb.hu">demenym@med.unideb.hu</a></td>
</tr>
<tr>
<td>Philippe Djian</td>
<td>CNRS/Université Paris Descartes</td>
<td><a href="mailto:philippe.djian@parisdescartes.fr">philippe.djian@parisdescartes.fr</a></td>
</tr>
<tr>
<td>Cedric Duval</td>
<td>University of Leeds</td>
<td><a href="mailto:C.Duval@leeds.ac.uk">C.Duval@leeds.ac.uk</a></td>
</tr>
<tr>
<td>Richard Eckert</td>
<td>University of Maryland School of Medicine</td>
<td><a href="mailto:reckert@umaryland.edu">reckert@umaryland.edu</a></td>
</tr>
<tr>
<td>Meddy El Alaoui</td>
<td>Centre Léon Berard</td>
<td><a href="mailto:Meddy.ELALAOU1@lyon.unicancer.fr">Meddy.ELALAOU1@lyon.unicancer.fr</a></td>
</tr>
<tr>
<td>Said El Alaoui</td>
<td>Covalab S.A.S.</td>
<td><a href="mailto:elalaoui@covalab.com">elalaoui@covalab.com</a></td>
</tr>
<tr>
<td>Rita Elek</td>
<td>University of Debrecen</td>
<td><a href="mailto:elek.rita@science.unideb.hu">elek.rita@science.unideb.hu</a></td>
</tr>
<tr>
<td>Eleonora Candi</td>
<td>University of Rome</td>
<td><a href="mailto:candi@uniroma2.it">candi@uniroma2.it</a></td>
</tr>
<tr>
<td>Shaun Alexander Fell</td>
<td>Aston University</td>
<td><a href="mailto:fellsa@aston.ac.uk">fellsa@aston.ac.uk</a></td>
</tr>
<tr>
<td>Lásló Fésüs</td>
<td>University of Debrecen</td>
<td><a href="mailto:fesus@med.unideb.hu">fesus@med.unideb.hu</a></td>
</tr>
<tr>
<td>Martin Griffin</td>
<td>Aston University</td>
<td><a href="mailto:m.griffin@aston.ac.uk">m.griffin@aston.ac.uk</a></td>
</tr>
<tr>
<td>Rhiannon Griffiths</td>
<td>Cardiff University</td>
<td><a href="mailto:griffithsrsm3@cardiff.ac.uk">griffithsrsm3@cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Christoph Hauser</td>
<td>University of Cologne, Medical Faculty</td>
<td><a href="mailto:christoph.hauser@uk-koeln.de">christoph.hauser@uk-koeln.de</a></td>
</tr>
<tr>
<td>Sandra Hauser</td>
<td>Helmholtz-Zentrum Dresden-Rossendorf</td>
<td><a href="mailto:s.hauser@hzdr.de">s.hauser@hzdr.de</a></td>
</tr>
<tr>
<td>Kiyotaka Hitomi</td>
<td>Nagoya University</td>
<td><a href="mailto:hitomi@ps.nagoya-u.ac.jp">hitomi@ps.nagoya-u.ac.jp</a></td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Email</td>
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</tr>
<tr>
<td>Samuel Hjorth-Jensen</td>
<td>Queensland University of Technology</td>
<td><a href="mailto:samuel.hjorthjensen@hdr.qut.edu.au">samuel.hjorthjensen@hdr.qut.edu.au</a></td>
</tr>
<tr>
<td>Boglárka Hurják</td>
<td>University of Debrecen</td>
<td><a href="mailto:hurjakbogi@gmail.com">hurjakbogi@gmail.com</a></td>
</tr>
<tr>
<td>Siiri Epp Iismaa</td>
<td>Victor Chang Cardiac Research Institute</td>
<td><a href="mailto:s.iismaa@victorchang.edu.au">s.iismaa@victorchang.edu.au</a></td>
</tr>
<tr>
<td>Károly Jambrovics</td>
<td>University of Debrecen</td>
<td><a href="mailto:jambrovics.karoly@med.unideb.hu">jambrovics.karoly@med.unideb.hu</a></td>
</tr>
<tr>
<td>Gail VW Johnson</td>
<td>University of Rochester</td>
<td><a href="mailto:gail_johnsonvoll@urmc.rochester.edu">gail_johnsonvoll@urmc.rochester.edu</a></td>
</tr>
<tr>
<td>Timothy Scott Johnson</td>
<td>UCB Biopharma / University of Sheffield</td>
<td><a href="mailto:tim.johnson@UCB.com">tim.johnson@UCB.com</a></td>
</tr>
<tr>
<td>János Kappelmayer</td>
<td>University of Debrecen</td>
<td><a href="mailto:kappelmayer@med.unideb.hu">kappelmayer@med.unideb.hu</a></td>
</tr>
<tr>
<td>Éva Katona</td>
<td>University of Debrecen</td>
<td><a href="mailto:ekatona@med.unideb.hu">ekatona@med.unideb.hu</a></td>
</tr>
<tr>
<td>Jeffrey Keílkor</td>
<td>University of Ottawa</td>
<td><a href="mailto:jkeillor@uottawa.ca">jkeillor@uottawa.ca</a></td>
</tr>
<tr>
<td>Robert Kiraly</td>
<td>University of Debrecen</td>
<td><a href="mailto:kiralyr@med.unideb.hu">kiralyr@med.unideb.hu</a></td>
</tr>
<tr>
<td>Beáta Kiss</td>
<td>University of Debrecen</td>
<td><a href="mailto:kissbea@med.unideb.hu">kissbea@med.unideb.hu</a></td>
</tr>
<tr>
<td>Agnes Klusoczki</td>
<td>University of Debrecen</td>
<td><a href="mailto:klusoczkiagnes@gmail.com">klusoczkiagnes@gmail.com</a></td>
</tr>
<tr>
<td>Soichi Kojima</td>
<td>RIKEN Center for Life Science Technologies</td>
<td><a href="mailto:skojima@postman.riken.go.jp">skojima@postman.riken.go.jp</a></td>
</tr>
<tr>
<td>Ilma Korponay-Szabó</td>
<td>University of Debrecen</td>
<td><a href="mailto:ilma.korponay-szabo@uta.fi">ilma.korponay-szabo@uta.fi</a></td>
</tr>
<tr>
<td>Krisztina Köröskényi</td>
<td>University of Debrecen</td>
<td><a href="mailto:kkriszti@med.unideb.hu">kkriszti@med.unideb.hu</a></td>
</tr>
<tr>
<td>Kinga Lénárt</td>
<td>University of Debrecen</td>
<td><a href="mailto:kinga.lenart@hotmail.com">kinga.lenart@hotmail.com</a></td>
</tr>
<tr>
<td>Reik Loeser</td>
<td>Helmholt-Zentrum Dresden Rossendorf</td>
<td><a href="mailto:r.loeser@hzdr.de">r.loeser@hzdr.de</a></td>
</tr>
<tr>
<td>András Mádi</td>
<td>University of Debrecen</td>
<td><a href="mailto:madi@med.unideb.hu">madi@med.unideb.hu</a></td>
</tr>
<tr>
<td>Bernadett Mártus</td>
<td>University of Debrecen</td>
<td><a href="mailto:jakob.bernadett@med.unideb.hu">jakob.bernadett@med.unideb.hu</a></td>
</tr>
<tr>
<td>Nicole McNeil</td>
<td>University of Ottawa</td>
<td><a href="mailto:nhatchet@uottawa.ca">nhatchet@uottawa.ca</a></td>
</tr>
<tr>
<td>Kapil Mehta</td>
<td>UT MD anderson Cancer Center</td>
<td><a href="mailto:Kapilmeh@gmail.com">Kapilmeh@gmail.com</a></td>
</tr>
<tr>
<td>László Muszbek</td>
<td>University of Debrecen</td>
<td><a href="mailto:muszbek@med.unideb.hu">muszbek@med.unideb.hu</a></td>
</tr>
<tr>
<td>László Nagy</td>
<td>University of Debrecen</td>
<td><a href="mailto:lnagy@me.com">lnagy@me.com</a></td>
</tr>
<tr>
<td>Zsuzsanna Zita</td>
<td>University of Debrecen</td>
<td><a href="mailto:zsorosz@med.unideb.hu">zsorosz@med.unideb.hu</a></td>
</tr>
<tr>
<td>Orosz</td>
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<tr>
<td>Ralf Pasternack</td>
<td>ZEDIRA</td>
<td><a href="mailto:pasternack@zedira.com">pasternack@zedira.com</a></td>
</tr>
<tr>
<td>Mauro Piacentini</td>
<td>University of Rome &quot;Tor Vergata&quot;</td>
<td><a href="mailto:mauro.piacentini@uniroma2.it">mauro.piacentini@uniroma2.it</a></td>
</tr>
<tr>
<td>Markus Pietsch</td>
<td>University of Cologne, Medical Faculty</td>
<td><a href="mailto:markus.pietsch@uk-koeln.de">markus.pietsch@uk-koeln.de</a></td>
</tr>
<tr>
<td>Xian-Yang Qin</td>
<td>RIKEN Center for Life Science Technologies</td>
<td><a href="mailto:xyqin@riken.jp">xyqin@riken.jp</a></td>
</tr>
<tr>
<td>Tibor Saghy</td>
<td>University of Debrecen</td>
<td><a href="mailto:saghy.tibor@med.unideb.hu">saghy.tibor@med.unideb.hu</a></td>
</tr>
<tr>
<td>Zsolt Sarang</td>
<td>University of Debrecen</td>
<td><a href="mailto:sarang@med.unideb.hu">sarang@med.unideb.hu</a></td>
</tr>
<tr>
<td>Halime Siğinç</td>
<td>Yeditepe University</td>
<td><a href="mailto:halime_ilhan@hotmail.com">halime_ilhan@hotmail.com</a></td>
</tr>
<tr>
<td>Sneha Singh</td>
<td>University Clinic Bonn</td>
<td><a href="mailto:sneha.gupta@ukbonn.de">sneha.gupta@ukbonn.de</a></td>
</tr>
<tr>
<td>Sándor Sipka</td>
<td>University of Debrecen</td>
<td><a href="mailto:sipka@iiibel.dote.hu">sipka@iiibel.dote.hu</a></td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Email</td>
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<tr>
<td>Éva Sivadó</td>
<td>Covalab, Centre Léon-Bérard</td>
<td><a href="mailto:e.sivado@covalab.com">e.sivado@covalab.com</a></td>
</tr>
<tr>
<td>Ludvig M Sollid</td>
<td>University of Oslo</td>
<td><a href="mailto:l.m.sollid@medisin.uio.no">l.m.sollid@medisin.uio.no</a></td>
</tr>
<tr>
<td>Laura Somodi</td>
<td>University of Debrecen</td>
<td><a href="mailto:somodilaura@gmail.com">somodilaura@gmail.com</a></td>
</tr>
<tr>
<td>Huifang Sun</td>
<td>McGill University</td>
<td><a href="mailto:huifang.sun@mail.mcgill.ca">huifang.sun@mail.mcgill.ca</a></td>
</tr>
<tr>
<td>Zsuzsa Szabo</td>
<td>University of Debrecen</td>
<td><a href="mailto:hallizsuzs@hotmail.com">hallizsuzs@hotmail.com</a></td>
</tr>
<tr>
<td>Timea Szekely</td>
<td>University of Debrecen</td>
<td><a href="mailto:timcsl0826@gmail.com">timcsl0826@gmail.com</a></td>
</tr>
<tr>
<td>Zsuzsanna Szondy</td>
<td>University of Debrecen</td>
<td><a href="mailto:szondy@med.unideb.hu">szondy@med.unideb.hu</a></td>
</tr>
<tr>
<td>Elisa Tonoli</td>
<td>Nottingham Trent University</td>
<td><a href="mailto:elisa.tonoli2015@my.ntu.ac.uk">elisa.tonoli2015@my.ntu.ac.uk</a></td>
</tr>
<tr>
<td>Daniel Torocsik</td>
<td>University of Debrecen</td>
<td><a href="mailto:dtorocsik@gmail.com">dtorocsik@gmail.com</a></td>
</tr>
<tr>
<td>Boglárka Tóth</td>
<td>University of Debrecen</td>
<td><a href="mailto:tothbogi@med.unideb.hu">tothbogi@med.unideb.hu</a></td>
</tr>
<tr>
<td>Shannon Tuberville</td>
<td>Cardiff University</td>
<td><a href="mailto:Turbervilles@cardiff.ac.uk">Turbervilles@cardiff.ac.uk</a></td>
</tr>
<tr>
<td>Bürge Ulukan</td>
<td>Yeditepe University</td>
<td><a href="mailto:ulukanburge@gmail.com">ulukanburge@gmail.com</a></td>
</tr>
<tr>
<td>Anne-Marie van Dam</td>
<td>VU University Medical Center</td>
<td><a href="mailto:amw.vandam@vumc.nl">amw.vandam@vumc.nl</a></td>
</tr>
<tr>
<td>Eli Verdeio</td>
<td>NTU</td>
<td><a href="mailto:eliverdee@metawolds.myzen.co.uk">eliverdee@metawolds.myzen.co.uk</a></td>
</tr>
<tr>
<td>Elisabetta AM Verderio</td>
<td>Nottingham Trent University</td>
<td><a href="mailto:elisabetta.verderio-edwards@ntu.ac.uk">elisabetta.verderio-edwards@ntu.ac.uk</a></td>
</tr>
<tr>
<td>Alexandra D. Vasilyeva</td>
<td>N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences</td>
<td><a href="mailto:ms.kadaver@mail.ru">ms.kadaver@mail.ru</a></td>
</tr>
<tr>
<td>Zhuo Wang</td>
<td>Aston University</td>
<td><a href="mailto:z.wangz10@aston.ac.uk">z.wangz10@aston.ac.uk</a></td>
</tr>
<tr>
<td>Robert Wodtke</td>
<td>Helmholtz-Zentrum Dresden-Rossendorf</td>
<td><a href="mailto:r.wodtke@hzdr.de">r.wodtke@hzdr.de</a></td>
</tr>
</tbody>
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