MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

January 2019 - September 2020

MUI-START Report

Forschungsservice und Innovation
Medical University of Innsbruck
January 2019 - September 2020

Content

1 Background and aim of the programme

MUI-START is the follow-up programme of the MFI (Medizinische Forschungsfonds Innsbruck) which ended in 2011.

The MUI-START programme is devised as a start-up fund for young scientists with the aim to offer them the opportunity of developing new project ideas, within the MUI research focuses, that could serve as basis for a successful subsequent application for external funding (e. g. FWF).

According to the present guidelines, eligible candidates must: 1) have a working contract with the Medical University of Innsbruck for the entire project duration, 2) have completed their doctoral studies, and 3) the applicant´s most recent degree (e.g. PhD) must have been completed no longer than eight years ago. Fully justified career breaks can be taken into account (e. g. parental leave). Professors and PIs of third-party funded (FWF, OeNB, FFG and EU) projects are not eligible. Applicants' track records must be commensurate with their academic age. However, two peer-reviewed international publications as first author are compulsory**.**

The guidelines of the programme have been substantially modified over the years to adapt to the high standards applied by external funding agencies (e. g. FWF). Since 2016, proposals undergo a three-step evaluation procedure: 1) selection of proposals by the MUI-START jury, 2) international peer-review of the pre-selected proposals, and 3) hearing of the shortlisted applicants by the MUI-START jury. Final decisions are based on the reviewers' scores, as well as the outcome of the interviews.

Moreover, since the seventh call (2016) the submission of an external funding proposal before the end of the funding period has become compulsory for all MUI-START grantees. Failure to submit such an application results in the cancellation of the last quarter of the MUI-START grant budget.

2 Overview of MUI-START calls

The first MUI-START call was announced in the summer 2010 and supported 42% of the submitted proposals. Since then, the approval rates have been oscillating from year to year (Table 1) depending on both the available budget and the quality of the submitted proposals. The funded projects from the 11th call will start in December 2020.

Table 1. Overview of all MUI-START calls

Year	Call	Proposals submitted	Proposals granted (Male/Female)	Funding rate	Total funding requested	Total funding granted
2010	1st	31	13 (7M/6F)	42%	€ 2.074.365,7	€ 667.054,80
2011	2nd	11	5 (2M/3F)	45%	€ 629.968,9	€ 173.171,00

2012	3rd	29	9 (4M/5F)	31%	€ 742.808,2	€ 240.000,00
2013	4th	28	14(11M/3F)	50%	€ 713.652,9	€ 323.484,70
2014	5th	31	12(4M/8F)	39%	€ 771.750,5	€ 260.826,60
2015	6th	28	8 (4M/4F)	28%	€ 711.035,4	€ 176.726,00
2016	7th	9	3 (1M/2F)	33%	€ 248.945,0	€ 85.000,00
2017	8th	15	7 (5M/2F)	47%	€ 365.189,3	€ 162.208,80
2018	9th	8	4 (1M/3F)	50%	€ 192.576,2	€ 113.766,30
2019	10th	9	0 (0M/0F)	0%	€ 529.947,8	€ 00,00
2020	11th	16	3 (1M/2F)	19%	€ 559.657,5	€ 101.454,10

3 MUI-START jury members and reviewers

The MUI START jury members are professors and associate professors at the Medical University of Innsbruck working in both basic as well as in clinical research fields. The jury members are chosen according to their expertise in a specific field of research. The composition of the jury is not fixed, but changes as a result of the variety of topics covered by the proposals submitted to a particular call.

The following jury members helped in the selection of the projects presented in this report. Their help and commitment is warmly acknowledged.

Univ.-Prof. Dr.rer.nat. Christine BANDTLOW	Institute of Neurobiochemistry
Univ.-Prof. Dr.rer.nat. Georg DECHANT	Joint Institution for Neuroscience
Univ.-Prof. Dr. Francesco FERRAGUTI	Institute of Pharmacology
Univ.-Prof. Dr.med. Elke GIZEWSKI MHBA	University Hospital for Neuroradiology
Univ.-Prof. Dr. Ludger HENGST	Institute of Medical Biochemistry
Univ.-Prof. Dr.med.univ. Susanne KASER	University Hospital for Internal Medicine I

Univ.-Prof. Dr. Alexandra LUSSER	Institute of Molecular Biology
Univ.-Prof. Dr.med.univ. Matthias SCHMUTH	University Hospital for Dermatology, Venerology and Allergology
Univ.-Prof. Dr.med.univ. Gert Mayer	University Hospital for Internal Medicine IV
Univ.-Prof. Dr.rer.nat. Patrizia STOITZNER	University Hospital for Dermatology, Venerology and Allergology
Univ.-Prof. Dr.med.univ. Günter WEISS	University Hospital for Internal Medicine II
ao. Univ.-Prof. Dr.med.univ. Johann WILLEIT	University Hospital for Neurology
Univ.-Prof. Dr.med. Johannes ZSCHOCKE PhD	Institute of Human Genetics

The tasks of the jury members comprise: 1) internal review of the proposals, 2) nomination of the reviewers at the suggestion of the MUI Research Office (FSI), and 3) presentation of proposals during the decision meeting.

The reviewers of the MUI-START projects are international experts active in their field of research. Usually two reviews per proposal are necessary to support the jury members decision process.

4 MUI-START Symposium and project evaluation

By accepting the MUI-START grant, the recipients commit themselves to take part in the annual MUI-START Symposium organized by the MUI Research Office (FSI). This event represents an ideal occasion for the MUI-START jury members to assess the progress achieved by the grant holders in their respective projects. Additionally, the symposium promotes a mentoring effect for the grant holders who profit from the expertise and advice of the jury members.

During the last MUI-START Symposium a total of 7 projects were evaluated. The results of this evaluation are summarized below and in Fig. 1.

In more than 70% of the cases, the project development was considered good or very good. The PI qualifications and the research environment were considered good or very good in 94% of the evaluated projects. However, despite these positive results the jury members judged that only two thirds of the projects could translate into an external funding application. This discrepancy is due to the fact that several PIs quit the MUI earlier than expected or immediately after the end of the funding period. In few other cases, the jury members thought it would be difficult to find a suitable funding programme to submit a proposal.

Links: 7th MUI-START Symposium Programme

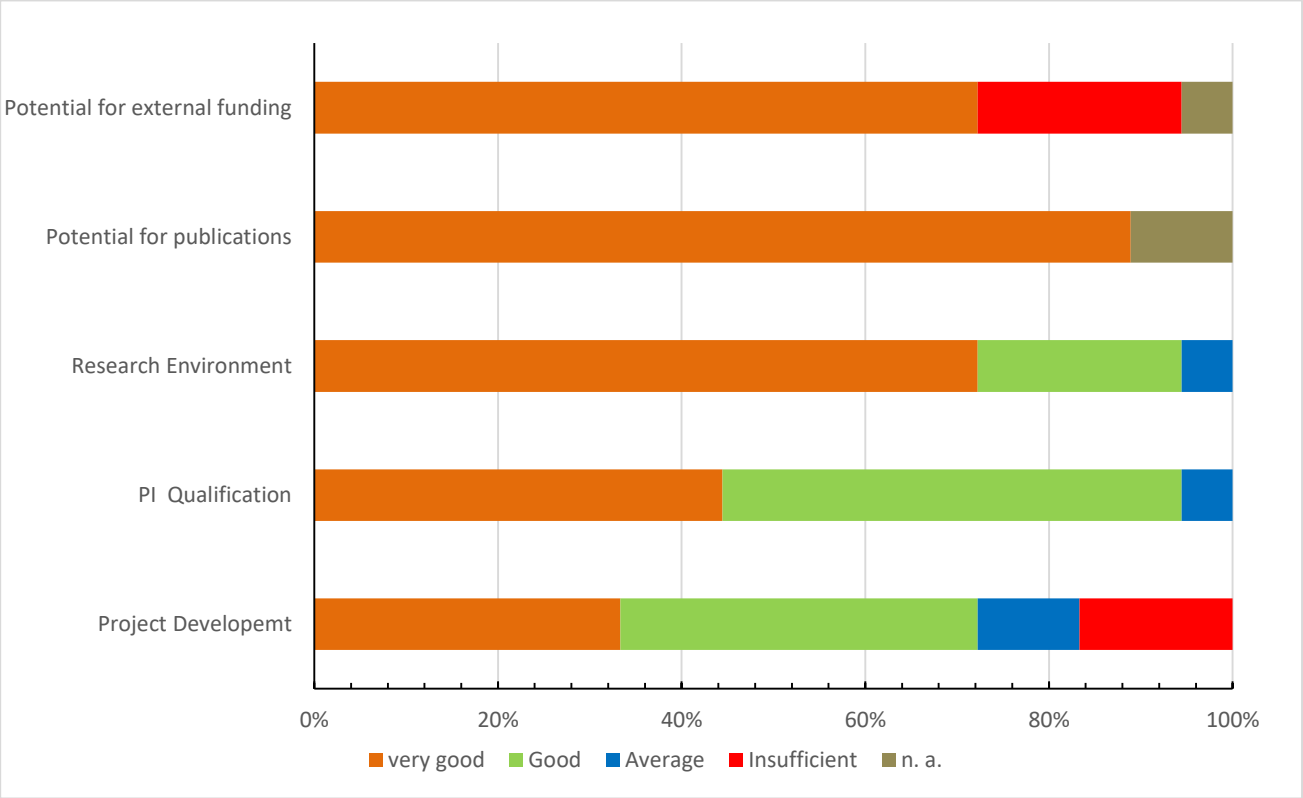

Fig. 1. Results of the evaluation of projects (7th and 8th Calls) during the MUI-START Symposium. n.a. no answer.

5 External funding granted to MUI-START grant recipients

As stated in the first section of this report the aim of the programme is to help young scientists develop new project ideas that could serve as a basis for a subsequent application for third-party funding.

So far, (status as of 30.09.2020) 65 MUI-START funded projects have closed. Nineteen PIs quit the MUI either before the planned end or immediately after the end of the project. Approximately 85 % (39) of the remaining PIs applied for external funding. Given the competitiveness of the current funding landscape, not all applications generated funding. Table 2 and Table 3 provide an overview of the funds acquired by the MUI-START grantees to date.

Table 2. FWF funded projects acquired by former MUI-START grant holders in the period covered by this annual report.

Applicant	Project Title	Funding Agency-Project Number	Duration (Months)	Funds Granted

Manfred Nairz	The iron storage protein ferritin in bacterial infection	FWF – P 33062	48	€ 399.429,56
Ramon Tasan	Role of the neurokinin B-expressing neurons in the bed nucleus of the stria terminalis	FWF – P 29952	36	€ 399.441,00
Bernhard Haubner	Investigating long non-coding RNA regulated pathways driving cardiac regeneration	FWF – I 4161	36	€ 206.892,00
Sebastian Reinstadler (Co-applicant)	Soluble Neprilysin in ST-elevation Myocardial Infarction	FWF – KLI 772	48	€ 188.118,00
Michaela Lackner	Intrinsic azole resistance in mucormycetes	FWF – P32329	48	€ 306.517,05
Markus Keller	Lipid peroxidation as driver of cardiolipin remodeling	FWF–P33333	36	€ 380.962,52
Kerstin Bellaire-Siegmund	Sox matters!	FWF – TAI88	18	€ 127.027,74

Table 3. Additional third party funding acquired by former MUI-START grant holders in the period covered by the annual report.

Applicant	Project Title	Funding Agency	Duration (Months)	Funds Granted

Sebastian Reinstadler	Soluble Neprilysin in ST-elevation myocardial infarction: Release Kinetics, Cardiac Remodeling and Future Cardiovascular Events	Österrichische Kardiologische Gesselschaft	24	€ 48.880,00

6 Publications acknowledging the MUI-START programme

This section lists publications arising from the MUI-START projects that ended between January 2019 and end of September 2020. A list of publications of projects that finished before this period can be found in the former MUI-START reports.

Reinstadler SJ, Klug G, Feistritzer HJ, Kofler M, Pernter B, Göbel G, Henninger B, Müller S, Franz WM, Metzler B. Prognostic value of left ventricular global function index in patients after ST-segment elevation myocardial infarction. Eur Heart J Cardiovasc Imaging. 2016 Feb;17(2):169-76. doi: 10.1093/ehjci/jev129.

Reinstadler SJ, Feistritzer HJ, Reindl M, Klug G, Mayr A, Mair J, Jaschke W, Metzler B. Combined biomarker testing for the prediction of left ventricular remodelling in ST-elevation myocardial infarction. Open Heart. 2016 Sep 20;3(2):e000485. doi: 10.1136/openhrt-2016-000485.

Reindl M, Reinstadler SJ, Feistritzer HJ, Tiller C, Mayr A, Klug G, Metzler B. Heart rate and left ventricular adverse remodelling after ST-elevation myocardial infarction. Int J Cardiol. 2016 Sep 15;219:339-44. doi: 10.1016/j.ijcard.2016.06.046.

Reindl M, Reinstadler SJ, Feistritzer HJ, Mueller L, Koch C, Mayr A, Theurl M, Kirchmair R, Klug G, Metzler B. Fibroblast growth factor 23 as novel biomarker for early risk stratification after ST-elevation myocardial infarction. Heart. 2017 Jun;103(11):856-862. doi: 10.1136/heartjnl-2016-310520.

Reindl M, Reinstadler SJ, Feistritzer HJ, Niess L, Koch C, Mayr A, Klug G, Metzler B. Persistent T-wave inversion predicts myocardial damage after ST-elevation myocardial infarction. Int J Cardiol. 2017 Aug 15;241:76-82. doi: 10.1016/j.ijcard.2017.03.164.

Feistritzer HJ, Klug G, Reinstadler SJ, Reindl M, Niess L, Nalbach T, Kremser C, Mayr A, Metzler B. Prognostic Value of Aortic Stiffness in Patients After ST-Elevation

Myocardial Infarction. J Am Heart Assoc. 2017 Sep 8;6(9):e005590. doi: 10.1161/JAHA.117.005590.

Reinstadler SJ, Kronbichler A, Reindl M, Feistritzer HJ, Innerhofer V, Mayr A, Klug G, Tiefenthaler M, Mayer G, Metzler B. Acute kidney injury is associated with microvascular myocardial damage following myocardial infarction. Kidney Int. 2017 Sep;92(3):743-750. doi: 10.1016/j.kint.2017.02.016.

Reindl M, Reinstadler SJ, Feistritzer HJ, Theurl M, Basic D, Eigler C, Holzknecht M, Mair J, Mayr A, Klug G, Metzler B. Relation of Low-Density Lipoprotein Cholesterol With Microvascular Injury and Clinical Outcome in Revascularized ST-Elevation Myocardial Infarction. J Am Heart Assoc. 2017 Oct 10;6(10):e006957. doi: 10.1161/JAHA.117.006957.

Reindl M, Reinstadler SJ, Feistritzer HJ, Klug G, Tiller C, Mair J, Mayr A, Jaschke W, Metzler B. Relation of inflammatory markers with myocardial and microvascular injury in patients with reperfused ST-elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2017 Oct;6(7):640-649. doi: 10.1177/2048872616661691.

Reindl M, Reinstadler SJ, Tiller C, Kofler M, Theurl M, Klier N, Fleischmann K, Mayr A, Henninger B, Klug G, Metzler B. ACEF score adapted to ST-elevation myocardial infarction patients: The ACEF-STEMI score. Int J Cardiol. 2018 Aug 1;264:18-24. doi: 10.1016/j.ijcard.2018.04.017.

Reindl M, Feistritzer HJ, Reinstadler SJ, Mueller L, Tiller C, Brenner C, Mayr A, Henninger B, Mair J, Klug G, Metzler B. Thyroid-stimulating hormone and adverse left ventricular remodeling following ST-segment elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2019 Dec;8(8):717-726. doi: 10.1177/2048872618770600.

Tiller C, Reindl M, Reinstadler SJ, Holzknecht M, Schreinlechner M, Peherstorfer A, Hein N, Lechner I, Mayr A, Klug G, Metzler B. Complete versus simplified Selvester QRS score for infarct severity assessment in ST-elevation myocardial infarction. BMC Cardiovasc Disord. 2019 Dec 9;19(1):285. doi: 10.1186/s12872-019-1230-0.

Reindl M, Holzknecht M, Tiller C, Lechner I, Schiestl M, Simma F, Pamminger M, Henninger B, Mayr A, Klug G, Bauer A, Metzler B, Reinstadler SJ. Impact of infarct location and size on clinical outcome after ST-elevation myocardial infarction treated by

primary percutaneous coronary intervention. Int J Cardiol. 2020 Feb 15;301:14-20. doi: 10.1016/j.ijcard.2019.11.123.

Tiller C, Reindl M, Holzknecht M, Innerhofer L, Wagner M, Lechner I, Mayr A, Klug G, Bauer A, Metzler B, Reinstadler SJ. Relationship between admission Q waves and microvascular injury in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Int J Cardiol. 2019 Dec 15;297:1-7. doi: 10.1016/j.ijcard.2019.10.009.

Tiller C, Reindl M, Holzknecht M, Klapfer M, Beck A, Henninger B, Mayr A, Klug G, Reinstadler SJ, Metzler B. Biomarker assessment for early infarct size estimation in ST-elevation myocardial infarction. Eur J Intern Med. 2019 Jun;64:57-62. doi: 10.1016/j.ejim.2019.03.001.

Reinstadler SJ, Reindl M, Tiller C, Holzknecht M, Klug G, Metzler B. Obesity paradox in ST-elevation myocardial infarction: is it all about infarct size? Eur Heart J Qual Care Clin Outcomes. 2019 Apr 1;5(2):180-182. doi: 10.1093/ehjqcco/qcy042.

Csanaky K, Hess MW, Klimaschewski L. Membrane-Associated, Not Cytoplasmic or Nuclear, FGFR1 Induces Neuronal Differentiation. Cells. 2019 Mar 14;8(3):243. doi: 10.3390/cells8030243.

Gollmann-Tepeköylü C, Graber M, Pölzl L, Nägele F, Moling R, Esser H, Summerer B, Mellitzer V, Ebner S, Hirsch J, Schäfer G, Hackl H, Cardini B, Oberhuber R, Primavesi F, Öfner D, Bonaros N, Troppmair J, Grimm M, Schneeberger S, Holfeld J, Resch T. Toll-like receptor 3 mediates ischaemia/reperfusion injury after cardiac transplantation. Eur J Cardiothorac Surg. 2020 May 1;57(5):826-835. doi: 10.1093/ejcts/ezz383.

Demetz E, Tymoszuk P, Hilbe R, Volani C, Haschka D, Heim C, Auer K, Lener D, Zeiger LB, Pfeifhofer-Obermair C, Boehm A, Obermair GJ, Ablinger C, Coassin S, Lamina C, Kager J, Petzer V, Asshoff M, Schroll A, Nairz M, Dichtl S, Seifert M, von Raffay L, Fischer C, Barros-Pinkelnig M, Brigo N, Valente de Souza L, Sopper S, Hirsch J, Graber M, Gollmann-Tepeköylü C, Holfeld J, Halper J, Macheiner S, Gostner J, Vogel GF, Pechlaner R, Moser P, Imboden M, Marques-Vidal P, Probst-Hensch NM, Meiselbach H, Strauch K, Peters A, Paulweber B, Willeit J, Kiechl S, Kronenberg F, Theurl I, Tancevski I, Weiss G. The haemochromatosis gene Hfe and Kupffer cells control LDL cholesterol homeostasis and impact on atherosclerosis development. Eur Heart J. 2020 Oct 21;41(40):3949-3959. doi: 10.1093/eurheartj/ehaa140.

Haubner BJ, Schuetz T, Penninger JM. Cardiac regeneration in a newborn: what does this mean for future cardiac repair research? Expert Rev Cardiovasc Ther. 2018 Mar;16(3):155-157. doi: 10.1080/14779072.2018.1438268.

Publications acknowledging the MUI-START programme that were not listed in the former MUI-START report (January 2017- December 2018)

Bauer I, Gross S, Merschak P, Kremser L, Karahoda B, Bayram ÖS, Abt B, Binder U, Gsaller F, Lindner H, Bayram Ö, Brosch G, Graessle S. RcLS2F - A Novel Fungal Class 1 KDAC Co-repressor Complex in *Aspergillus nidulans*. Front Microbiol. 2020 Feb 4;11:43. doi: 10.3389/fmicb.2020.00043.

Bauer I, Misslinger M, Shadkchan Y, Dietl AM, Petzer V, Orasch T, Abt B, Graessle S, Osherov N, Haas H. The Lysine Deacetylase RpdA Is Essential for Virulence in *Aspergillus fumigatus*. Front Microbiol. 2019 Dec 4;10:2773. doi: 10.3389/fmicb.2019.02773.

Bauer I, Pidroni A, Bayram Ö, Brosch G, Graessle S. Single-Step Enrichment of a TAP-Tagged Histone Deacetylase of the Filamentous Fungus Aspergillus nidulans for Enzymatic Activity Assay. J Vis Exp. 2019 May 1;(147):10.3791/59527. doi: 10.3791/59527.

Messner F, Fischer AC, Runggaldier E, Sprung S, Müller J, Eiter S, Gantschnigg A, Zelger B, Zelger B, Wolfram D, Öfner D, Hautz T, Schneeberger S. Mechanical Irritation in Vascularized Composite Tissue Allotransplantation Triggers Localized Skin Rejection. Transplantation. 2020 May;104(5):956-969. doi: 10.1097/TP.0000000000003075.

Oemer G, Koch J, Wohlfarter Y, Alam MT, Lackner K, Sailer S, Neumann L, Lindner HH, Watschinger K, Haltmeier M, Werner ER, Zschocke J, Keller MA. Phospholipid Acyl Chain Diversity Controls the Tissue-Specific Assembly of Mitochondrial Cardiolipins. Cell Rep. 2020 Mar 24;30(12):4281-4291.e4. doi: 10.1016/j.celrep.2020.02.115.

Huber A, Oemer G, Malanovic N, Lohner K, Kovács L, Salvenmoser W, Zschocke J, Keller MA, Marx F. Membrane Sphingolipids Regulate the Fitness and Antifungal Protein Susceptibility of *Neurospora crassa*. Front Microbiol. 2019 Apr 11;10:605. doi: 10.3389/fmicb.2019.00605.

Koch J, Lackner K, Wohlfarter Y, Sailer S, Zschocke J, Werner ER, Watschinger K, Keller MA. Unequivocal Mapping of Molecular Ether Lipid Species by LC-MS/MS in

Plasmalogen-Deficient Mice. Anal Chem. 2020 Aug 18;92(16):11268-11276. doi: 10.1021/acs.analchem.0c01933.

Gerner RR, Macheiner S, Reider S, Siegmund K, Grabherr F, Mayr L, Texler B, Moser P, Effenberger M, Schwaighofer H, Moschen AR, Kircher B, Oberacher H, Zeiser R, Tilg H, Nachbaur D. Targeting NAD immunometabolism limits severe graft-versus-host disease and has potent antileukemic activity. Leukemia. 2020 Jul;34(7):1885-1897. doi: 10.1038/s41375-020-0709-0.

7 The MUI-START programme in numbers (status as of 30 September 2020)

- **78 Proposals granted / 65 Projects completed**
- **40 Male PIs / 38 Female PIs**
- **104 Publications acknowledging the MUI-START programme**
- **€ 2,0 Mio granted by the MUI START programme**
- **€ 6,4 Mio funds acquired by former MUI-START grant holders**

Additionally, 55% of PIs of closed projects now have a permanent position at the MUI or at the Tirol Kliniken. Another 29% of PIs quit the MUI and got positions in other research institutions or at pharmaceutical or high-tech companies. The remaining scientists are still working at the MUI as project collaborators or hold non-permanent positions.

8 MUI-START final reports

The principal investigators of the MUI-START funded projects are responsible for the content of their respective final reports.

S. Reinstadler - Prognostic significance of copeptin after ST-elevation myocardial infarction: Insights from cardiac magnetic resonance imaging

Institute for Internal Medicine III

6th Funding period

Project duration: 01.12.2015 – 30.08.2019

Summary

ST-segment elevation myocardial infarction (STEMI) is a feared manifestation of coronary artery disease that is caused by abrupt occlusion of a coronary artery resulting to myocardial ischemia and myocardial cell death. A rapid restoration of coronary flow in the occluded artery is the cornerstone of acute therapy for STEMI. As recommended by contemporary guidelines, primary percutaneous coronary intervention (PPCI) is the preferred reperfusion strategy if performed in a timely fashion. The implementation of PPCI has contributed to a decrease in mortality related to STEMI during the last years (1). However, the risk for recurrent cardiovascular events beyond the acute phase of STEMI remains considerable with significant differences across different subgroups of patients (2). It is important to emphasis that the risk of recurrent events decreases with time. Therefore, early knowledge of the individual risk of adverse events in survivors of STEMI is important and may help to improve patient management, implement novel treatment strategies and design future clinical trials with the aim of further optimizing outcome in these patients. Although risk stratification tools that employ traditional risk factors, such as TIMI risk score, are easy to apply, discrimination abilities are only moderate. Research efforts have therefore been directed to develop better tools for optimized risk assessment including biomarker testing during the acute phase of STEMI. Currently, several biomarkers, such as high-sensitivity troponin t(3), N-terminal pro-B-type natriuretic peptide(4), high- sensitivity c-reactive protein(5) and copeptin (6) are investigated for their usefulness for improving risk prediction in patients with STEMI. We have previously shown that single time point copeptin concentrations measured between day 1 and day 3 after ST-elevation myocardial infarction (STEMI) are associated with cardiac magnetic resonance (CMR) markers of adverse outcome (myocardial function, infarct size, microvascular obstruction)(6). Accordingly, copeptin might improve risk stratification of STEMI patients. However, copeptin rapidly decreases within the first days after infarction and therefore the most appropriate time point for the measurement of copeptin to risk stratify these patients' remains to be determined. We therefore aim to compare the use of multiple versus single time point copeptin measurements for the prediction of systolic dysfunction and myocardial damage as visualized by CMR at baseline, 4 months, and 12 months after primary percutaneous coronary intervention (p-PCI) in patients with acute STEMI. Moreover, we aim to evaluate the predictive value other biomarkers (high-sensitivity troponin t [hs-cTnT], N-terminal pro- B-type natriuretic peptide [NT-proBNP], and high-sensitivity c-reactive protein [hs-CRP]). So far, we could enroll the target study population of 100 patients with STEMI treated with PPCI. The median age of the overall study population is 57 years. Approximately 20% of the study population are females. Cardiovascular risk factor distribution (hypertension, diabetes, hyperlipidemia) is well comparable with current literature. All patients underwent a complete baseline CMR investigation for infarct characterization. However, follow-up CMR investigations are not completed yet. Concerning baseline CMR parameters, median left ventricular ejection fraction is 55% and median infarct size 16% of left

ventricular myocardial mass. Microvascular obstruction was found in 50% of patients. The following biomarker levels were measured so far: hs-TnT, NT-proBNP and hs-CRP at baseline. Median hs-cTnT levels are: 105 ng/L on admission, 3323 ng/L at 6 hours, 4027 ng/L at 12 hours, 2815 ng/L at 24 hours, and 2390 ng/L at 48 hours after STEMI. Median NT-proBNP levels are: 124 ng/L on admission, 476 ng/ at 6 hours, 788 ng/L at 12 hours, 1022 ng/L at 24 hours, and 1095 ng/L at 48 hours after STEMI. Median hs-CRP levels are: 0.2 mg/dL on admission, 0.5 mg/dL at 6 hours, 0.8 mg/dL at 12 hours, 1.3 mg/dL at 24 hours, and 2.8 mg/dL at 48 hours after STEMI. Taken together, baseline results regarding CMR markers of myocardial function and infarct severity are well comparable with contemporary STEMI cohorts. Furthermore, three biomarkers could be measured at baseline and correlation analysis of these biomarker concentrations with CMR parameters has already provided important insights into their association with infarct severity and their potential as prognostic markers after STEMI (please see publications below for further details). Moreover, further interesting biomarkers could be measured in this established cohort of patients. Continuation of this project will allow us to provide further insights into the relationship between serially measured biomarkers (those mentioned above as well as novel biomarkers not mentioned at the beginning of the project) and CMR markers of adverse outcome in patients after acute STEMI.

Reference list:

1. Jernberg T, Johanson P, Held C, Svennblad B, Lindback J, Wallentin L. Association between adoption of evidence-based treatment and survival for patients with ST-elevation myocardial infarction. Jama. 2011 Apr 27;305(16):1677-84. PubMed PMID: 21521849. Epub 2011/04/28. eng.

2. Yamashita Y, Shiomi H, Morimoto T, Yaku H, Furukawa Y, Nakagawa Y, et al. Cardiac and Noncardiac Causes of Long-Term Mortality in ST-Segment-Elevation Acute Myocardial Infarction Patients Who Underwent Primary Percutaneous Coronary Intervention. Circulation Cardiovascular quality and outcomes. 2017 Jan;10(1). PubMed PMID: 28073848. Epub 2017/01/12. eng.

3. Reinstadler SJ, Feistritzer HJ, Klug G, Mair J, Tu AM, Kofler M, et al. High-sensitivity troponin T for prediction of left ventricular function and infarct size one year following ST-elevation myocardial infarction. International journal of cardiology. 2016 Jan 1;202:188-93. PubMed PMID: 26397408. Epub 2015/09/24.

4. Reinstadler SJ, Feistritzer HJ, Reindl M, Klug G, Metzler B. Utility of NT-proBNP in predicting infarct scar and left ventricular dysfunction at a chronic stage after myocardial infarction. European journal of internal medicine. 2016 Apr;29:e16-8. PubMed PMID: 26776924. Epub 2016/01/19.

5. Reindl M, Reinstadler SJ, Feistritzer HJ, Klug G, Tiller C, Mair J, et al. Relation of inflammatory markers with myocardial and microvascular injury in patients with reperfused ST-elevation myocardial infarction. European heart journal Acute cardiovascular care. 2017 Oct;6(7):640-9. PubMed PMID: 27440935. Epub 2016/07/22.

6. Reinstadler SJ, Klug G, Feistritzer HJ, Mayr A, Harrasser B, Mair J, et al. Association of copeptin with myocardial infarct size and myocardial function after ST

segment elevation myocardial infarction. Heart. 2013 Oct;99(20):1525-9. PubMed PMID: 23697651. Epub 2013/05/24.

Publications issued from this project

Reinstadler SJ, Klug G, Feistritzer HJ, Kofler M, Pernter B, Göbel G, Henninger B, Müller S, Franz WM, Metzler B. Prognostic value of left ventricular global function index in patients after ST-segment elevation myocardial infarction. Eur Heart J Cardiovasc Imaging. 2016 Feb;17(2):169-76. doi: 10.1093/ehjci/jev129.

Reinstadler SJ, Feistritzer HJ, Reindl M, Klug G, Mayr A, Mair J, Jaschke W, Metzler B. Combined biomarker testing for the prediction of left ventricular remodelling in ST-elevation myocardial infarction. Open Heart. 2016 Sep 20;3(2):e000485. doi: 10.1136/openhrt-2016-000485.

Reindl M, Reinstadler SJ, Feistritzer HJ, Tiller C, Mayr A, Klug G, Metzler B. Heart rate and left ventricular adverse remodelling after ST-elevation myocardial infarction. Int J Cardiol. 2016 Sep 15;219:339-44. doi: 10.1016/j.ijcard.2016.06.046.

Reindl M, Reinstadler SJ, Feistritzer HJ, Mueller L, Koch C, Mayr A, Theurl M, Kirchmair R, Klug G, Metzler B. Fibroblast growth factor 23 as novel biomarker for early risk stratification after ST-elevation myocardial infarction. Heart. 2017 Jun;103(11):856-862. doi: 10.1136/heartjnl-2016-310520.

Reindl M, Reinstadler SJ, Feistritzer HJ, Niess L, Koch C, Mayr A, Klug G, Metzler B. Persistent T-wave inversion predicts myocardial damage after ST-elevation myocardial infarction. Int J Cardiol. 2017 Aug 15;241:76-82. doi: 10.1016/j.ijcard.2017.03.164.

Feistritzer HJ, Klug G, Reinstadler SJ, Reindl M, Niess L, Nalbach T, Kremser C, Mayr A, Metzler B. Prognostic Value of Aortic Stiffness in Patients After ST-Elevation Myocardial Infarction. J Am Heart Assoc. 2017 Sep 8;6(9):e005590. doi: 10.1161/JAHA.117.005590.

Reinstadler SJ, Kronbichler A, Reindl M, Feistritzer HJ, Innerhofer V, Mayr A, Klug G, Tiefenthaler M, Mayer G, Metzler B. Acute kidney injury is associated with microvascular myocardial damage following myocardial infarction. Kidney Int. 2017 Sep;92(3):743-750. doi: 10.1016/j.kint.2017.02.016.

Reindl M, Reinstadler SJ, Feistritzer HJ, Theurl M, Basic D, Eigler C, Holzknecht M, Mair J, Mayr A, Klug G, Metzler B. Relation of Low-Density Lipoprotein Cholesterol With Microvascular Injury and Clinical Outcome in Revascularized ST-Elevation Myocardial Infarction. J Am Heart Assoc. 2017 Oct 10;6(10):e006957. doi: 10.1161/JAHA.117.006957.

Reindl M, Reinstadler SJ, Feistritzer HJ, Klug G, Tiller C, Mair J, Mayr A, Jaschke W, Metzler B. Relation of inflammatory markers with myocardial and microvascular injury in patients with reperfused ST-elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2017 Oct;6(7):640-649. doi: 10.1177/2048872616661691.

Reindl M, Reinstadler SJ, Tiller C, Kofler M, Theurl M, Klier N, Fleischmann K, Mayr A, Henninger B, Klug G, Metzler B. ACEF score adapted to ST-elevation myocardial infarction patients: The ACEF-STEMI score. Int J Cardiol. 2018 Aug 1;264:18-24. doi: 10.1016/j.ijcard.2018.04.017.

Reindl M, Feistritzer HJ, Reinstadler SJ, Mueller L, Tiller C, Brenner C, Mayr A, Henninger B, Mair J, Klug G, Metzler B. Thyroid-stimulating hormone and adverse left ventricular remodeling following ST-segment elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2019 Dec;8(8):717-726. doi: 10.1177/2048872618770600.

Tiller C, Reindl M, Reinstadler SJ, Holzknecht M, Schreinlechner M, Peherstorfer A, Hein N, Lechner I, Mayr A, Klug G, Metzler B. Complete versus simplified Selvester QRS score for infarct severity assessment in ST-elevation myocardial infarction. BMC Cardiovasc Disord. 2019 Dec 9;19(1):285. doi: 10.1186/s12872-019-1230-0.

Reindl M, Holzknecht M, Tiller C, Lechner I, Schiestl M, Simma F, Pamminger M, Henninger B, Mayr A, Klug G, Bauer A, Metzler B, Reinstadler SJ. Impact of infarct location and size on clinical outcome after ST-elevation myocardial infarction treated by primary percutaneous coronary intervention. Int J Cardiol. 2020 Feb 15;301:14-20. doi: 10.1016/j.ijcard.2019.11.123.

Tiller C, Reindl M, Holzknecht M, Innerhofer L, Wagner M, Lechner I, Mayr A, Klug G, Bauer A, Metzler B, Reinstadler SJ. Relationship between admission Q waves and microvascular injury in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Int J Cardiol. 2019 Dec 15;297:1-7. doi: 10.1016/j.ijcard.2019.10.009.

Tiller C, Reindl M, Holzknecht M, Klapfer M, Beck A, Henninger B, Mayr A, Klug G, Reinstadler SJ, Metzler B. Biomarker assessment for early infarct size estimation in ST-elevation myocardial infarction. Eur J Intern Med. 2019 Jun;64:57-62. doi: 10.1016/j.ejim.2019.03.001.

Reinstadler SJ, Reindl M, Tiller C, Holzknecht M, Klug G, Metzler B. Obesity paradox in ST-elevation myocardial infarction: is it all about infarct size? Eur Heart J Qual Care Clin Outcomes. 2019 Apr 1;5(2):180-182. doi: 10.1093/ehjqcco/qcy042.

External funding

The following external funding applications which are related to the topic of biomarkers investigated in the MUI-START project have been accepted:

Acute Kidney Injury in reperfused ST- elevation myocardial infarction. (TWF-2018-1-6). Amount granted: € 36.160,00.

Research grant from the Austrian Society of Cardiology, “Soluble Neprilysin in ST-elevation myocardial infarction: Release Kinetics, Cardiac Remodeling and Future Cardiovascular Events” Amount granted: € 48.880,00

As co-applicant: Soluble Neprilysin in ST-elevation Myocardial Infarction (FWF- KLI772). Amount granted: 188.118,00 €.

Miscellaneous

Congress presentations:

- Annual meeting of the “Austrian society of cardiology” 2016, 2017 and 2018
- European Society of Cardiology Congress2017
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Prizes:

- “Werner-Klein-Award 2017” for translational research - Acute kidney injury is associated with microvascular myocardial damage following myocardial infarction. Kidney Int. 2017 Sep;92(3):743-750.

K. Csanaky - Influence of subcellular targeting of light-inducible opto-FGFR1 on neuronal differentiation

Institute of Neuroanatomy

7th Funding period

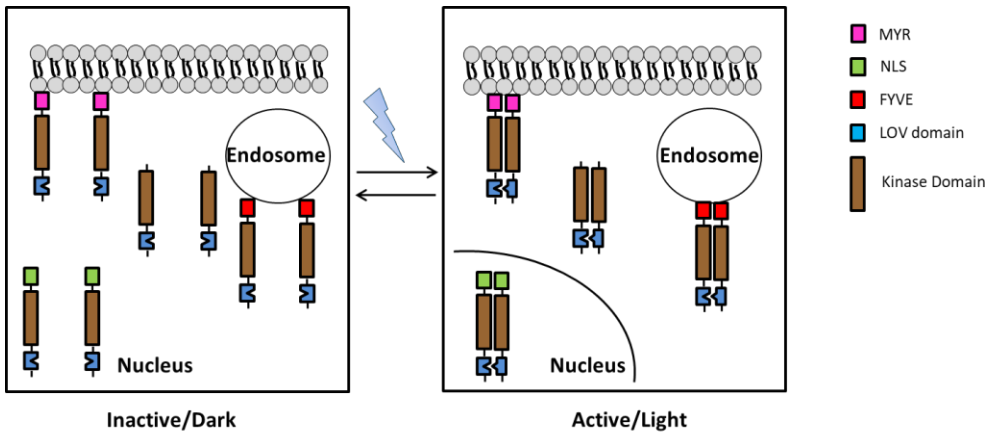
Project duration: 01.10.2017 – 30.09.2020

Summary

The intracellular transport of fibroblast growth factor receptor type 1 (FGFR1) results in differential activation of various signaling pathways which play a key role in neuronal differentiation and axon growth (Philos Trans R Soc Lond B Biol Sci 2006, 361(1473):1575-92). The extent of the stimulation of the major signaling pathways involved in neuronal differentiation and axon outgrowth is crucially dependent on the amount and localization of activated kinase domains, and it determines the functional and morphological outcome (Eur J Cell Biol 2012, 91(2):129-38; Semin Cell Dev Biol 2016, 53:155-67). These results have been obtained by using pharmacological inhibitors or mutant receptors.

Optogenetic approaches are ideal tools to control and manipulate cellular signaling in spatial and temporal precision. Members of the receptor tyrosine kinase family (TrkA and TrkB) showed light induced neurite outgrowth of PC12 cells (Nat Commun 2014, 5: 4057; ACS Synth Biol 2018, (7):1685- 1693). Janovjak et al. constructed a membrane anchored chimeric opto-fibroblast growth factor receptor (opto-FGFR1). The catalytic domain of the receptor was fused to an algal light-oxygen- voltage-sensing (LOV) domain, and short pulses of blue light induced LOV domain dimerization resulted in transphosphorylation of two receptor kinase molecules (EMBO J 2014, 33(15):1713-26). In 2015, in collaboration with this research group, we engineered cytosolic-, endosomal-, and nuclear- opto-FGFR1s. These constructs are seen in Fig. 1.

Fig. 1: Design of the light-controlled opto-FGFR1s and their molecular architecture: spatial relation of opto-FGFR1s to surface membrane, endosomes and nucleus, their activation/dimerization and the sequences of the different FGFR1 coding genes. The



artificial mem-opto- FGFR1 is anchored to the plasma membrane with an N-terminal myristoylation signal (MYR) followed by the KD (kinase domain) and LOV domain. Cyto-opto-FGFR1s contain no localization signal, and they consist of only KD and LOV

domains. Endosomal opto-FGFR1 consists of two phosphatidylinositol 3-phosphate [PI(3)P]-binding FYVE finger domains followed KD and LOV domain. Besides KD and LOV domain, 3 NLS signals are inserted into nucl-opto-FGFR1.

The goal of the project was 1) to analyze the function of the different subcellular targeted opto-mFGFR1 molecules with regard to inducing signaling and neuronal differentiation, 2) to generate stable PC12 cell lines expressing the four different mVenus labeled opto-mFGFR1 constructs, 3) to visualize subcellular activation of signaling pathways (combining FRET-based fluorescent sensors of ERK activity with the opto-mFGFR1 constructs to measure the different activation of cytoplasmic and nuclear ERK in live cell imaging mode), and 4) to stimulate FGFR1 molecules selectively at the plasma membrane, in endosomes or in the nucleus for a well-defined time period and to determine the short- and long-term effects of spatially controlled FGFR1 signaling on neuronal differentiation.

Methods used in this project: Cultures of transiently transfected HEK293 and PC12 cells were used in this study. The localization of different opto-constructs was detected by fluorescent and electron microscopy. The activation of downstream kinase signaling was followed by Western blot and by immunocytochemistry. Fluorescent microscopy was applied to detect neuronal differentiation, and it was quantitatively measured with Metamorph software.

Results:

Characterization of HEK293 and PC12 cells: Since the PC12 cells showed low, approximately 10% transfection efficiency, they were not a promising candidate for the control experiments. Therefore, HEK293 cells were used to analyze the localization and the function the different opto-FGFR1 constructs by fluorescent- and electron microscopy and Western blot. For all quantitative signaling assays on HEK293 cells, the levels of active FGFR1 (pFGFR1) and the control protein (GAPDH) were determined to normalize for different transfection efficiencies.

Opto-FGFR1s expressing stable cell lines were not generated, but instead PC12 cell lines enriched with mV-mem-opto-FGFR1-, mV-cyto-opto-FGFR1- or mV-nucl-opto-FGFR1-transfected cells were obtained. With this method the transfection efficiency increased (appr. 20%), but this was still not enough to perform Western blot analysis on PC12 cells, therefore we applied immunocytochemistry to detect the activation of signaling proteins on this cell line.

The endogenous level of FGFRs was determined by RT-PCR, and it revealed that all the four FGFR mRNAs were endogenously expressed in both HEK293 and PC12 cells. In HEK293 cells, FGFR1 mRNA was detected at considerably higher levels than FGFR2, -3, or -4 mRNA, while in PC12 cells the expression of FGFR1 mRNA was low.

Localization of opto-FGFR1s in HEK293 cells: Immunofluorescence microscopy showed the correct subcellular localization of our constructs (Fig. 2).

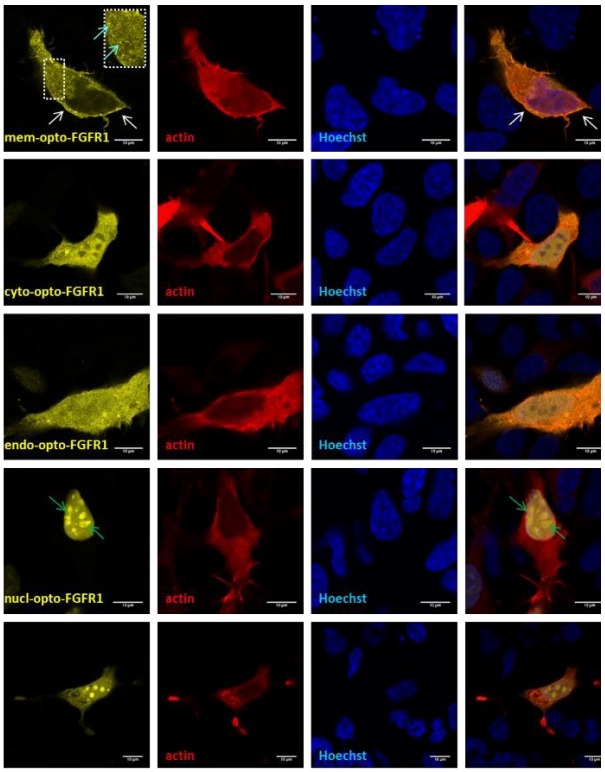


Fig. 2. Immunofluorescence microscopy of HEK293 cells co-transfected with mV-opto-FGFR1s and LifeAct-mCherry. Mem- opto-FGFR1 is observed at the surface membrane (white arrows) and in endosomes (see inset, cyan arrows) indicating endosomal receptor internalization. Cyto-opto-FGFR1 transfected cells reveal diffuse yellow fluorescence in the cytoplasm and nucleus of transfected cells. Endo-opto-FGFR1 showed similar diffuse fluorescence as the cytosolic construct. Nucl-opto-FGFR1 is located in the nucleus only (presumptive nuclear speckle domains indicated by green arrows). Inhibition of the importin-β machinery with 25 μM importazole results in diffuse cytoplasmic yellow fluorescence of the nucl-opto-FGFR1. LifeAct-mCherry was used to visualize the filamentous actin cytoskeleton. Fixed cell nuclei are stained with Hoechst. Scale bars: 10 μm.

The distribution of endosomal opto-FGFR1 receptor was diffuse or spotted (see Fig. 3), therefore we performed co-localization experiments with early endosome antigen 1 (EEA1).

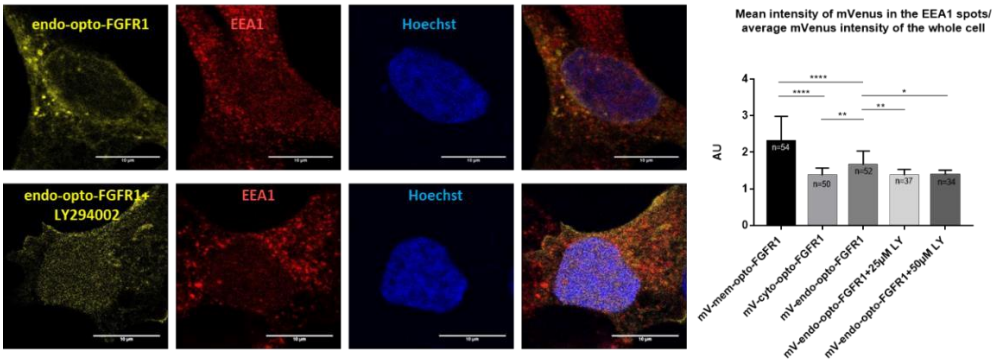


Fig. 3. Co-localization of endo-opto-FGFR1 and early endosome antigen 1 (EEA1). The endosomal constructs contain two PI(3)P-binding FYVE zinc fingers, therefore PI3K inhibitor (LY294002) should abolish the binding to endosomal membranes. Endosomal-opto-FGFR1 transfected HEK293 cells were pretreated with 25µM LY294002 and stained with EEA1. Quantification results of Imaris spot analysis showed higher mean fluorescence intensities of mVenus in the EEA1 positive vesicles of the endo-opto-FGFR1 transfected cells compared to the cytosolic construct. LY294002 treatment significantly decreased the yellow fluorescent intensities in the EEA1 positive vesicles Scale bars: 10 µm.

The endocytic pathway of the membrane-bound opto-FGFR1 and the localization of the endosomal opto-FGFR1 construct were analyzed by immunoelectron microscopy. Unfortunately, the yield of the endosomal construct was too low, therefore we did not use it for further experiments.

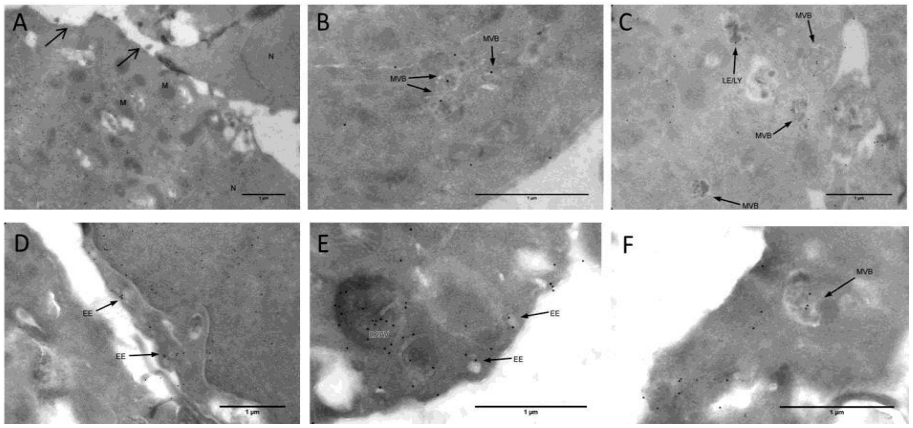


Fig. 4. Ultrastructural localization of mVenus in transfected HEK293 cells. A-C) Mem-opto-FGFR1 receptors (indicated by immunogold particles) are found at membranes, particularly at the cellular surface (arrows in A) as well as in mature multivesicular bodies (large, single membrane delimited organelles containing internal vesicles (arrows in B) which may fuse with lysosomes (electron-dense organelle with multilammelar structures (arrows in C). D-F) Endo-opto-FGFR1 is localized in early endosomes, just below the surface (pear shape with one intraluminar vesicle and with a long nose, D), in lysosomes (E) and in MVBs (F). N = nucleus, M = mitochondrium, MVB = multivesicular body, EE = early endosmone, LE = late endosome, LY = lysosome. Scale bars: 1 µm.

Activation of FGFR1 signaling by light on HEK293 and P12 cells: Activation of ERK but not AKT was observed in cells expressing membrane-associated and cytoplasmic opto-FGFR1 after blue light stimulation (Fig. 5). Induction of ERK was observed for 15 minutes, and it was completely blocked by MEK/ERK pathway inhibitor (PD98059) treatment (Fig. 6).

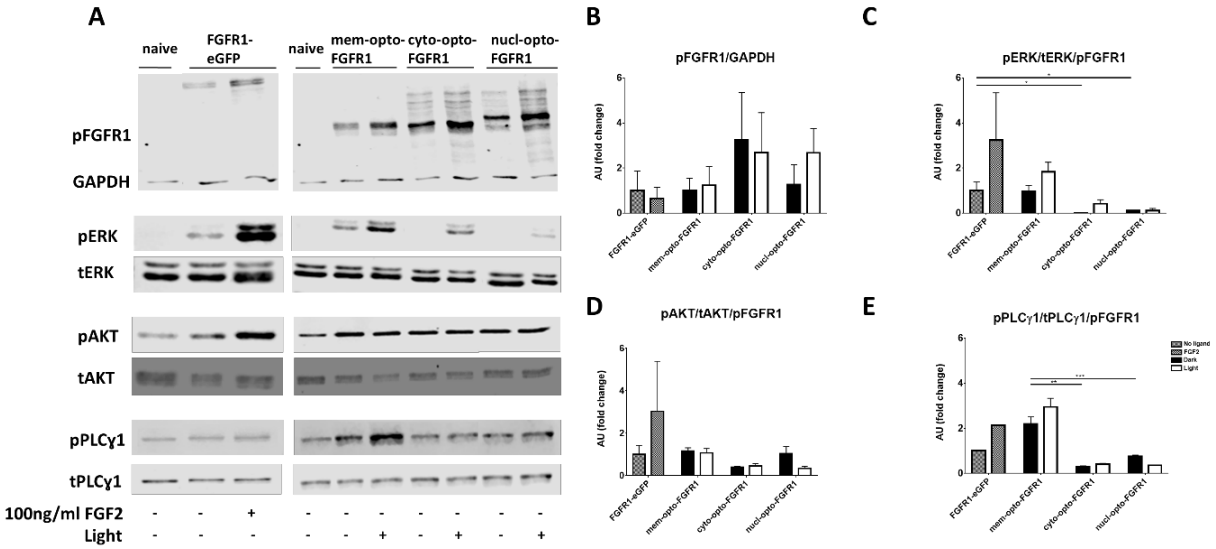


Fig. 5. Immunoblot analysis of light and FGF2-induced activation of key signaling molecules in HEK293 cells. (A) Phosphorylation of FGFR1, ERK, AKT and PLCɣ1. Band intensities of (B) FGFR1, (C) ERK, (D) AKT, and (E) PLCɣ1 phosphorylation were densitometrically quantified and the ratios were plotted relative to FGFR1-eGFP overexpressing cells. Results are calculated from three independent experiments and presented as mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001.

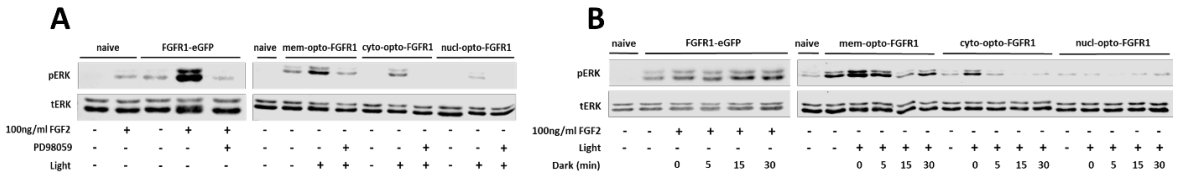


Fig. 6. Phosphorylation of ERK in response to ligand and blue light stimulation in HEK293 cells expressing FGFR1-eGFP or opto-FGFR1s. (A) Induction of ERK was completely blocked by PD98059 treatment. (B) Blue light stimulation for 5 min following opto-mem-FGFR1 or cyto- opto-FGFR1 transfection significantly elevated pERK levels which was reduced to pre-stimulation levels within 15 min. In contrast to mem- opto-FGFR1, light-induced ERK activation decreased shortly after cessation of the light stimulus in cyto-opto-FGFR1 transfected cells.

PC12 cells: ERK phosphorylation is known to trigger a conformational change of EKAR and increases FRET between EGFP and mRFP1 fluorescence proteins. Therefore, measuring the activation of ERK signaling after blue light stimulation by live cell imaging with a fluorescent sensor was part of this project. An elevated acceptor/donor fluorescence ratio was observed in our experiments after NGF treatment. Unfortunately, we could not find a reliable setting for the opto-FGFR1 activation with confocal microscopy, since the cytoplasmic EKARs are available in EGFP/mRFP and Cerulean/Venus tagged acceptor-donor pairs, and we could not combine them with our mVenus tagged opto-FGFR1 constructs. A non-labelled opto-FGFR1 expressing stable cell line would be an option in this setting.

Immunocytochemistry showed intense pERK immunolabeling in the cytoplasm, but only weak fluorescence in the nucleus 55 minutes after blue light stimulation (Fig.7).

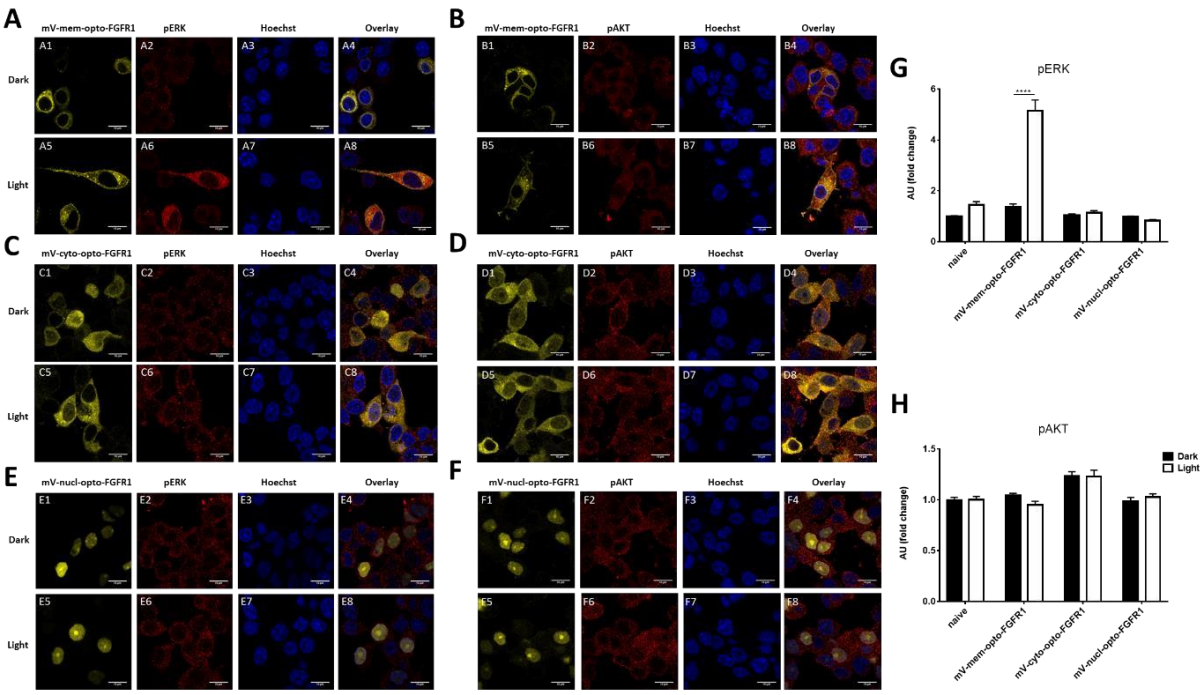


Fig. 7. Light-induced ERK and AKT activation in mV-opto-FGFR1s transfected PC12 cells. Although mV-mem-opto-FGFR1 transfected cells exhibit short neurite extensions (sprouts) in the dark, blue light induces neuronal differentiation with long, slender neurites in transfected cells. (A) Cytoplasmic pERK levels are significantly increased following light stimulation of mV-mem-opto-FGFR1 transfected cells, while non-transfected cells show no changes. (C, E) The pERK level is unchanged after stimulation of mV-cyto-and nucl-opto-FGFR1 transfected cells. (B, D, F) Fluorescence intensities of pAKT signals are similar in mV-opto-FGFR1s transfected and non-transfected cells in the dark and no changes are observed after blue light stimulation. Images are acquired using the same laser intensity for both dark and light in each fluorescent channel and presented without adjusting contrast or subtracting background. (G, H) Quantification of average fluorescence intensities. Results are calculated from two independent experiments and presented as mean ± SEM (30 ≤ n ≥ 60), ****p < 0.0001. Scale bars indicate 10 µm

Neuronal differentiation of PC12 cells by membrane, cytosolic and nuclear opto-FGFR1: The neurite outgrowth was followed over 24, 48 and 72h after NGF, FGF2 and light stimulation. Similar to NGF and FGF2 treatment, our cells showed prominent blue light-induced neuronal differentiation after 48 hours, and their density was optimal for single cell analysis, because the cells did not show overlapping.

The number of neurites extending from the cell body of mV-mem-opto-FGFR1-transfected cells increased significantly after blue light stimulation, and the neurites were significantly longer compared to NGF and FGF2 treatment (Fig. 8).

The opto-FGFR1 transfected cells were also treated with MEK/ERK pathway inhibitor for 2 hours before light stimulation. The light induced neuronal differentiation was completely blocked in the presence of the inhibitor.

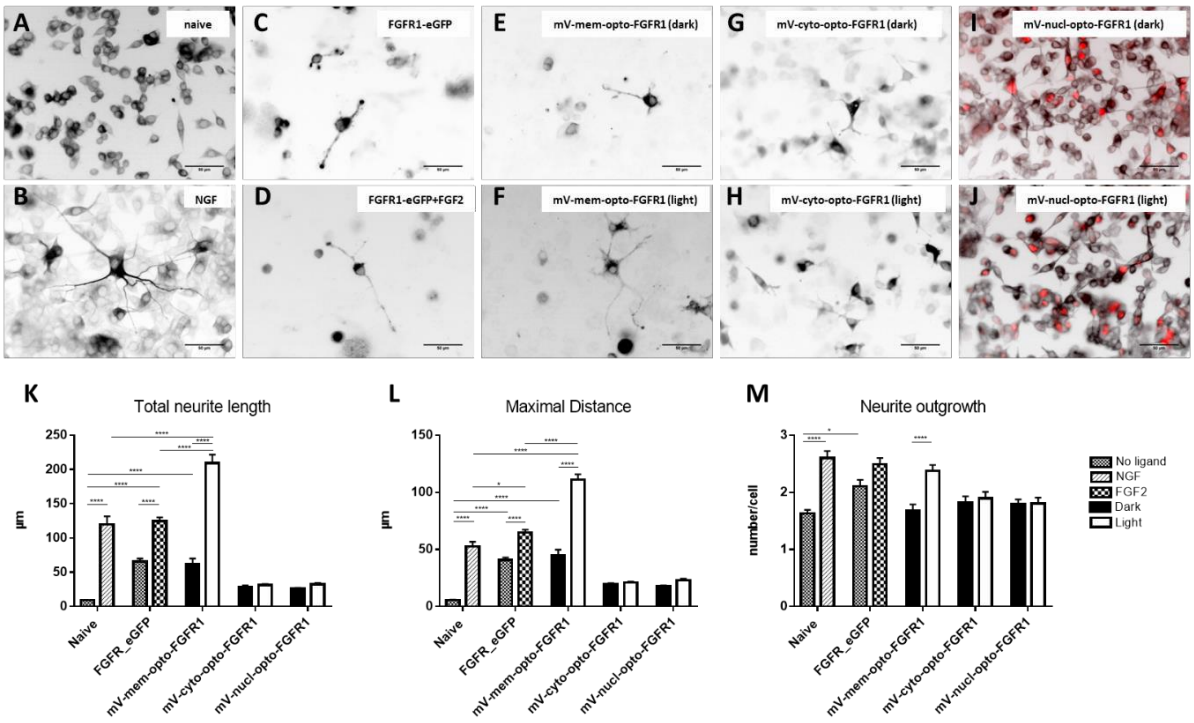


Fig. 8. Ligand and light induced neurite growth by PC12 cells. (A-J) Inverted immunofluorescence images following neuron-specific class III β-tubulin staining to identify neurites of mV-opto-FGFR1 transfected cells (red nuclei indicate nucl-opto-FGFR1 transfected cells in I/J). Repetitive 5 min ON and 55 min OFF cycles were used for stimulation over 48 h. Scale bars: 50 µm. (K-M) Quantification of morphological parameters (total neurite outgrowth, longest process, and number of processes per cell). Results are calculated from three independent experiments and presented as mean ± SEM (50 ≤ n ≥ 100), *p < 0.05, ****p < 0.0001.

Conclusion and Outlook: Our study provides evidence that FGFR1-dependent signaling pathways can be controlled and manipulated optogenetically, which makes it possible to study the subcellular receptor activation with spatial and temporal precision. Fully functional signaling platforms are only formed in association with cell membranes. Active RTKs apparently recruit different adaptors and scaffold proteins in plasma membranes as compared to endosomal and other membranes which exhibit different curvatures and phosphatidylinositol compositions.

We demonstrated that only membrane-bound opto-FGFR1 constructs are capable of activating the ERK pathway sufficiently to induce neuronal cell differentiation. Our observations on FGFR1 signaling pathway and the experiments on neuronal differentiation after blue light stimulation of FGFR1 are in harmony. Stimulation of FGFR1, in contrast to TrkA, exerts a significantly stronger influence on the ERK than on the AKT pathway. This is consistent with earlier studies of our group (Int Rev Neurobiol 2013, 108:137-71) demonstrating that activation of ERK machinery is predominantly implicated in elongation after injury, while PI3K/AKT signaling regulates branching.

Optogenetic stimulation of FGFR1 as a new experimental technique was installed in our laboratory in connection with this project. Further promising experiments can be initiated on the basis of this technique, such as adapting it to primary neurons and investigating the effect of opto- FGFR1 stimulation on neuronal morphology and function in organotypic hippocampal cultures.

Publications issued by this project

Csanaky K, Hess MW, Klimaschewski L. Membrane-Associated, Not Cytoplasmic or Nuclear, FGFR1 Induces Neuronal Differentiation. Cells. 2019 Mar 14;8(3):243. doi: 10.3390/cells8030243.

External funding

None

Miscellaneous

Under supervision of the applicant, Maximilian Klein completed his master thesis in the course of this project.

M. Lackner - Azole resistance in mucormycetes, understanding underlying molecular mechanisms

Institute of Hygiene and Medical Microbiology

8th Funding period

Project duration: 01.10.2017 - 30.11.2018

Summary

Objective:

The aim of this project is to provide a basis for understanding and studying molecular mechanisms of azole resistance (voriconazole, itraconazole, isavucoanzole, and posaconazole) in *Mucor circinelloides, Rhizopus microsporus*, and *Rhizopus arrhizus*.

Results:

The pilot studies and data generated using the MUI START grant have been used to strengthen the FWF proposal. A patch of methods and assays were set up to provide enough basis for a FWF grant. The following preliminary data could be added to the FWF proposal:

Strains. We now possess a comprehensive collection of *M. circinelloides* clinical isolates (N = 54) from Europe and India.

Susceptibility. The collection has been tested using the EUCAST method for susceptibility to short-tailed azoles (FLC, triadimenol, VCZ)[15], a medium-tailed azole (ISA) (unpublished data), and long-tailed azoles (PCZ, ITC). We have evaluated several standardized susceptibility methods and found the EUCAST method gave reproducible susceptibility profiles [27].

Resistance genes. Whole genome data are available for *M. circinelloides f. lusitanicus* CBS 277.49 at NCBI (https://www.ncbi.nlm.nih.gov). Full gene sequences of the two CYP51 paralogs and the cognate NADPH-cytochrome P450 reductase are available online for *M. circinelloides* (loci HMPREF1544_03888.1, HMPREF1544_08704.1, and HMPREF1544_05946 (OAD00099.1). We have PCR amplified, sequenced, and characterized the two CYP51 paralogs of *M. circinelloides* and identified the SNPs that appear responsible for intrinsic short-tailed azole-resistance in *mucormycetes.* We have optimized a method for sequencing *M. circinelloides* CYP51 F1 and F5 genes (unpublished data).

Structural analysis of LDM. In November 2018, Lackner will undertake a four-month research stay (funded by a Royal Society of NZ Catalyst Fund Seeding grant PI: Brian Monk) in Monk's laboratory to gain hands on experience with the construction of recombinant strains overexpressing functional *Rhizopus arrhizus* LDM (RaLDM) F1 and F5 (and with their cognate reductase), the biochemical characterization of these LDMs, and their purification. The purified recombinant RaLDM F1 and F5 in complexes

with VCZ and PCZ will be used in crystal trials and for structural analysis by X-ray crystallography (using the Australian Synchrotron). This preliminary study, to be carried out at Otago (November 2018-March 2019) using *R. arrhizus*, will provide important molecular and structural biology expertise.

Phenotype and Fitness. My laboratory has conducted phenotypic characterization (including: sporulation ability/efficiency, morphological characteristics, growth rates, biomass) together with fitness evaluation (including: temperature tolerance, oxygen stress, iron depletion) for a wide range of fungal pathogens.

Gene expression. Our established gene expression assay (real time quantitative PCR) has demonstrated both CYP51 F1 and F5 are expressed in drug free conditions. This assay will be used to quantify CYP51 F1 and F5 expression in drug free conditions, the presence of either voriconazole, posaconazole and isavuconazole at MIC_{50} (50% of the minimal inhibitory concentration) and MIC.

Virulence. The impact on virulence potential of *Mucor circinelloides* mutants will be studied in the *Galleria mellonella* model established by Ulrike Binder. Survival assays will be conducted and fungal burden determined using a novel quantitative real-time quantitative PCR assay (using partial mitochondrial *rnl* genes) recently established for murine experiments (data unpublished) and adapted for the *Galleria* model.

Mucor circinelloides model. Together with the Molina group the first batch of *Mucor circinelloides* mutants was generated. The Molina group generated F1 and F5 deletion mutants in be background of *Mucor circinelloides.*

Publications issued by this project

Data will be published together with the data of the FWF grant

External funding

Characterisation of intrinsic azole resistance in mucormycetes (FWF- P 32329). Amount granted: 306,517.05 €.

Miscellaneous

Students: 2019; Moja Zafran (ERASMUS student-Slovenia)

Prices: Paul Ehrlich Gesellschaft Poster Award 2019

Conference talks: XVIII EMA Congress, 2019, Warsaw, title: Intrinsic short-tailed azole resistance in mucormycetes

A. Pircher - Genetic and transcriptomic profiling of tumor endothelial cells (TECs) from bone marrow and peripheral blood of multiple myeloma patients

University Hospital for Internal Medicine V

8th Funding period

Project duration: 01.10.2017 – 30.03.2020

Summary

Scientific Background and objectives: Current anti-angiogenic therapies for cancer therapy destroy tumor vessels by blocking VEGF, with the ultimate goal to starve cancer cells. Anti-VEGF therapy has been approved for various cancers, including metastatic colorectal cancer (CRC) and non-small cell lung cancer (NSCLC). However, VEGF-targeted therapy poses major problems and showed no effect in prostate cancer (PCA) and hematologic malignancies claiming for alternative anti-angiogenic strategies, based on fundamentally different mechanisms. Therefore, the aim of the present translational study is to isolate tumor endothelial cells (TECs) and normal endothelial cells (NECs) from multiple myeloma patients, which had to be switch to PCA patients undergoing a radical prostatectomy as well as to perform multi-omic profiling (including targeted metabolomics, transcriptomics) to identify new targets for anti-angiogenic therapies. Recent advances in single cell RNA sequencing and due to the background of the applicant being involved in several single-cell RNA seq studies in NSCLC we further performed the first tumor microenvironmental characterization in PCA.

Unexpected Results and limitations: The primary submission of the grant included the analysis of TECs and NECs from multiple myeloma patients. Unfortunately, we were not successful to isolate and culture bone marrow derived ECs and therefore we moved to PCA. We tested several procedures to enrich for ECs (CD31+ selection, Ulex lectin selection) in multiple myeloma, nevertheless we had fibroblast overgrowth. The PCA investigations were performed together with Assoz. Prof. Dr. I. Heidegger Pircher.

Methods: We isolated NECs and TECs from 50 radical prostatectomy specimens. After successful enrichment of NECs/TECs by culturing and CD31 magnetic bead purification we confirmed endothelial cell phenotype by immune-fluorescence and FACS analysis. Next we analyzed cell proliferation using 3H-thymidine incorporation assay. Furthermore, we collected NECs and TECs for unbiased RNA sequencing. Furthermore, we performed from 4 PCA patients scRNA-seq with focus on the TME composition (together with A. Krogsdam and Z. Trajanoski). Immunhistochemical validation of genes identified by bulk-RNA seq is ongoing.

Results: NECs and TECs could be successfully isolated with an estimated success rate of 80%. Phonotypical investigations showed high CD31 positivity reflecting EC phenotype. Furthermore, NECs and TECs differed from morphological aspects (cell size, cell nuclei and junction's formation). In addition, TECs are hyper-proliferative compared to NECs reflecting are hyper-motile states. To further investigate the transcriptomic profile of TECs and NECs we performed bulk RNA Seq (in total 35

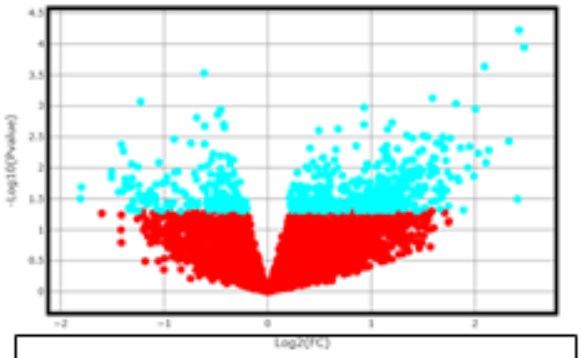


Figure1: Volcano plot showing differential regulated genes comparing cultured NEC and TEC

samples were sequenced (18 NEC and 17 TEC) and the analysis was performed with BIOMEX (published in Cancer Cell by Goveia et al. 2020, A. Pircher as Co-author). See Figure showing the Volcano plot with the most deregulated genes (PTGIR upregulated, CA8 downregulated).

We performed also pathway analysis where known tumor angiogenesis related pathways were upregulated. At the moment we are validating the target genes identified by bulk RNA-Seq with immunohistochemistry (Prof. Perner in Kiel). Analysis of the scRNA-Seq data is ongoing. First analysis showed that all stromal cell clusters are represented and we will deeper study the interaction of TEC and other stromal cell types.

Conclusion and Outlook: Here we show for the first time that human NECs and TECs can be isolated and cultured from fresh prostate tissue. First analysis of NECs and TECs show morphological and functional differences. Preliminary RNA-Seq analysis revealed difference in angiogenesis as well as immunoregulation pathways between TEC and NEC. The scRNA seq data will be evaluated soon and complemented with the bulk RNA Seq data.

Publications issued by this project

At the moment the presented data are not published yet. We have two papers in preparation. The MUI Start funding will be mentioned.

External funding

I submitted an OeNB grant. Unfortunately, the grant was not funded. Topic: Single cell mapping of treated and treatment naïve prostate cancer microenvironment

Miscellaneous

Diploma students (finished):

- Cand. Med. Roman Wiegele
- Cand. Med. Matthias Bayer

PhD student (ongoing): Sophia Daum MSc

Meetings: First results were presented at the ESUR meeting in Porto Lissabon: Comprehensive multi-omic profiling of tumor endothelial cells of prostate tissue October 2019European Urology Supplements 18(8):e3061; DOI: 10.1016/S1569-9056(19)33310-X

F. Plank - The PERSONALIZE-TAVI Project

University Hospital for Internal Medicine III

8th Funding period

Project duration: 01.01.2018 – 28.02.2020

Summary

Scientific background: Aortic stenosis is the most common valve disease in the industrial world and is associated with very high mortality rates if left untreated. In elderly and high-risk patients, transcatheter aortic valve has been established as an excellent treatment with comparative results as surgical conventional surgery. Nonetheless, this procedure relies solely on precise pre-procedural planning and can therefore be further enhanced using 3-D models to simulate implantation and prevent complications.

Methods: According to the study protocol, 35 patients underwent pre-procedural planning CT including ECG-gated cardiac reconstructions of the heart and the aorto-iliac arteries. Based on CT reconstructions, 3-dimensional constructions of the aortic root and adjacent structures were created digitally to plan and subsequently implant the transcatheter valve. Furthermore, replicas of the aortic valve including the aortic root and left ventricular outflow tract were printed. The aim of this process was to produce standardized prosthesis models to simulate individual size selection, landing zone, expansion level, anatomic details and potential procedural adjustments.

An additional post-procedural CT was performed to assess the implantation success and further evaluate computational fluid dynamics for flow patterns and shear stress.

A 3-Dimensional printing is performed in cooperation with the Management Center Innsbruck (MCI), further flow analytics are done with the health and life science university (UMIT).

First results: This first bedside approach of 3 dimensional planning of aortic valve implantation was set to guide proceduralists through individual anatomical differences and reduce complications. An initial approach using various software to overcome the conjunction between raw computed tomography data and 3 D printer compatible data has proven tedious (figure 1) and delayed the scheduled timeline. Crucial cornerstones (wall thickness, wall integrity, different material segmentation) were identified and difficult to overcome. However, a new cooperation with Materialise (Leuven, Belgium) has connected the missing data link and enabled first pilot prints. Additionally, we could present our project ´s blueprint at the inaugural symposium of the Austrian Platform for Personalized Medicine enhance our cooperation with the health and life science university (UMIT) and subsequent flow dynamic measurements.

At current, digital 3-D models were created for all 35 patients, 8 models were printed. Figure 2 shows an aortic root replica with white calcification.

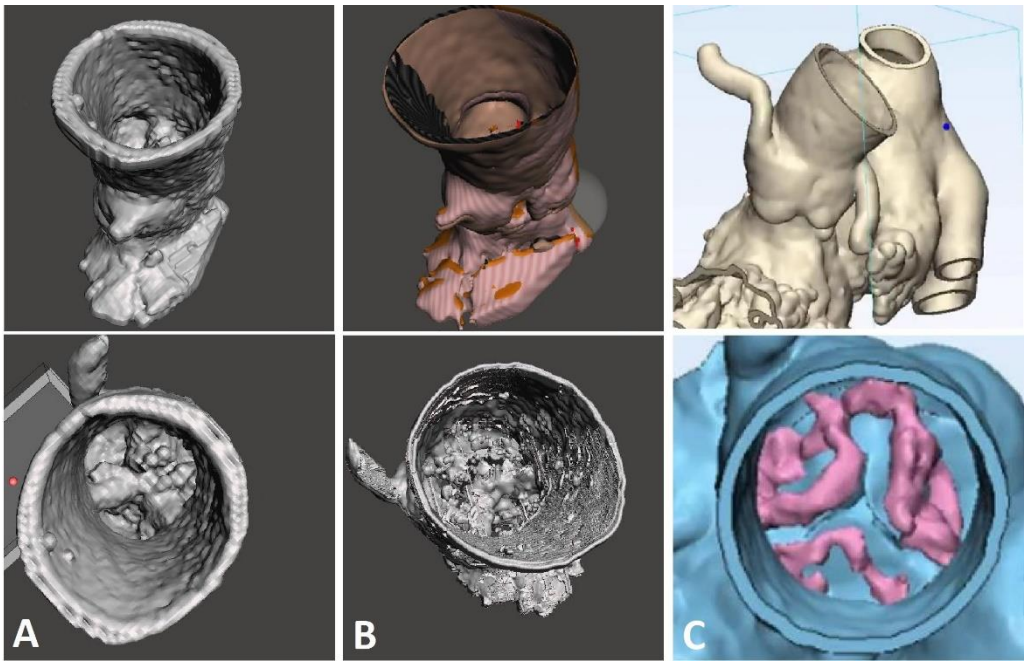


Figure 1. A 1st generation, B second generation and 3 third generation segmentation

Figure 2. 3-Dimensional Model

Next steps: With the new software solution, we are eager to advance the 3-D printed models to higher numbers and complete the initial sample of 35 patients. Further fluid dynamic analyses are in progress.

Publications issued by this project

None yet

External funding

TWF application in preparation, pending on the flow dynamics

Miscellaneous

Poster presentation: Austrian platform for personalized medicine: Inaugural Event and Scientific Symposium 19.10.2017

Diploma student: Supervision of diploma thesis Shana Heide.

E. Demetz - HFE deficiency critically affects cholesterol homeostasis

University Hospital for Internal Medicine II

8th Funding period

Project duration: 01.10.2017 – 31.03.2019

Summary

Aims: Imbalances of iron metabolism have been linked to the development of atherosclerosis. However, subjects with hereditary haemochromatosis have a lower prevalence of cardiovascular disease. The aim of our study was to understand the underlying mechanisms by combining data from genome-wide association study analyses in humans, CRISPR/Cas9 genome editing, and loss-of-function studies in mice.

Methods and results: Our analysis of the Global Lipids Genetics Consortium (GLGC) dataset revealed that single nucleotide polymorphisms (SNPs) in the haemochromatosis gene HFE associate with reduced low-density lipoprotein cholesterol (LDL-C) in human plasma. The LDL-C lowering effect could be phenocopied in dyslipidaemic ApoE-/- mice lacking Hfe, which translated into reduced atherosclerosis burden. Mechanistically, we identified HFE as a negative regulator of LDL receptor expression in hepatocytes. Moreover, we uncovered liver-resident Kupffer cells (KCs) as central players in cholesterol homeostasis as they were found to acquire and transfer LDL-derived cholesterol to hepatocytes in an Abca1-dependent fashion, which is controlled by iron availability.

Conclusion: Our results disentangle novel regulatory interactions between iron metabolism, KC biology and cholesterol homeostasis which are promising targets for treating dyslipidaemia but also provide a mechanistic explanation for reduced cardiovascular morbidity in subjects with haemochromatosis.

Publications issued by this project

Demetz E, Tymoszuk P, Hilbe R, Volani C, Haschka D, Heim C, Auer K, Lener D, Zeiger LB, Pfeifhofer-Obermair C, Boehm A, Obermair GJ, Ablinger C, Coassin S, Lamina C, Kager J, Petzer V, Asshoff M, Schroll A, Nairz M, Dichtl S, Seifert M, von Raffay L, Fischer C, Barros-Pinkelnig M, Brigo N, Valente de Souza L, Sopper S, Hirsch J, Graber M, Gollmann-Tepeköylü C, Holfeld J, Halper J, Macheiner S, Gostner J, Vogel GF, Pechlaner R, Moser P, Imboden M, Marques-Vidal P, Probst-Hensch NM, Meiselbach H, Strauch K, Peters A, Paulweber B, Willeit J, Kiechl S, Kronenberg F, Theurl I, Tancevski I, Weiss G. The haemochromatosis gene Hfe and Kupffer cells control LDL cholesterol homeostasis and impact on atherosclerosis development. Eur Heart J. 2020 Oct 21;41(40):3949-3959. doi: 10.1093/eurheartj/ehaa140.

External funding

FWF Stand Alone as co-applicant with Ivan Tancevski: „Lipid mediators as regulators of cholesterol homeostasis”. Unfortunately, the project did not receive funding. Another FWF application is currently being written.

Miscellaneous

The Austrian Atherosclerosis Society awarded the paper issued from the project as the best publication in the field of atherosclerosis research in 2020.

T. Resch - Toll-like receptor (TLR)-3 – a novel target for the prevention of ischemia-reperfusion injury in cardiac transplantation

University Hospital for Visceral, Transplant and Thoracic Surgery

8th Funding period

Project duration: 15.10.2017 – 14.10.2019

Summary

Objectives: Ischaemia and subsequent reperfusion during heart transplantation inevitably result in donor organ injury. Toll-like receptor (TLR)-3 is a pattern recognition receptor activated by viral and endogenous RNA released by injured cells. We hypothesized that ischaemia/reperfusion injury (IRI) leads to RNA release with subsequent TLR3 activation in transplanted hearts.

Methods: Human endothelial cells were subjected to IRI and treated with TLR3 agonist polyinosinic-polycytidylic acid or a TLR3/double-stranded RNA complex inhibitor. TLR3 activation was analysed using reporter cells. Gene expression profiles were evaluated via next-generation sequencing. Neutrophil adhesion was assessed in vitro. Syngeneic heart transplantation of wild-type or Tlr3-/- mice was performed following 9 h of cold ischaemia. Hearts were analysed for inflammatory gene expression, cardiac damage, apoptosis and infiltrating leucocytes.

Results: IRI resulted in RNA release with subsequent activation of TLR3. Treatment with a TLR3 inhibitor abrogated the inflammatory response upon IRI. In parallel, TLR3 stimulation caused activation of the inflammasome. Endothelial IRI resulted in TLR3-dependent adhesion of neutrophils. Tlr3-/- animals showed reduced intragraft and splenic messenger ribonucleic acid (mRNA) expression of proinflammatory cytokines, resulting in decreased myocardial damage, apoptosis and infiltrating cells. Tlr3 deficiency protected from cardiac damage, apoptosis and leucocyte infiltration after cardiac transplantation.

Conclusions: We uncover the release of RNA by injured cells with subsequent activation of TLR3 as a crucial pathomechanism of IRI. Our data indicate that TLR3 represents a novel target in the prevention of IRI in solid organ transplantation.

Outlook: In vivo results were generated by use of a syngeneic cardiac transplantation model. In a next step, the effects of TLR3 will be assessed in the setting of allotransplantation.

Publications issued by this project

Gollmann-Tepeköylü C, Graber M, Pölzl L, Nägele F, Moling R, Esser H, Summerer B, Mellitzer V, Ebner S, Hirsch J, Schäfer G, Hackl H, Cardini B, Oberhuber R, Primavesi F, Öfner D, Bonaros N, Troppmair J, Grimm M, Schneeberger S, Holfeld J,

Resch T. Toll-like receptor 3 mediates ischaemia/reperfusion injury after cardiac transplantation. Eur J Cardiothorac Surg. 2020 May 1;57(5):826-835. doi: 10.1093/ejcts/ezz383.

External funding

None yet

Miscellaneous

Congress presentations:

Toll-like receptor (TLR)-3 – a novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation. T. Resch, H. Esser, V. Mellitzer, S. Ebner, B. Cardini, R. Oberhuber, F. Primavesi, R. Moling, D. Öfner, J. Troppmair, S. Schneeberger, G. Schäfer, J. Holfeld, C. Tepeköylü; 31st Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics (Austrotransplant), Zell am See, Austria, October 2017

Transpl Int Volume 30 Suppl s3. [Oral presentation]

Toll-like receptor (TLR)-3 – a novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation. B. Summerer, H. Esser, V. Mellitzer, S. Ebner, B. Cardini, R. Oberhuber, R. Moling, S. Schneeberger, G. Schäfer, C. Tepeköylü, D. Öfner, J. Troppmair, **T. Resch:** 41. Seminar der Österreichischen Gesellschaft für Chirurgische Forschung, Schladming, Austria, November 2017. [Oral presentation]

Supervision of diploma students:

Diploma Thesis: “The effects of Toll like receptor 3 on experimental cardiac transplantation” Student: cand. Med. Rafael Moling

Prizes:

Young Investigator Award: basic science, Austrian Society of Transplantation, Zell am See, Austria, 2017; “Toll-like receptor (tlr)-3 -- a novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation'”, 1.5 T€

Award of the Austrian Society of Surgical Research, Schladming, Austria, 2017; Toll-like receptor (TLR)-3 - a novel target for the prevention of ischemia-reperfusion injury in solid organ transplantation”

B. Haubner - The Role of miRNA 6240 during Neonatal Cardiac Regeneration

University Hospital for Internal Medicine III

8th Funding period

Project duration: 01.10.2017 – 31.03.2020

Summary

In 2011 Porrello et al. reported a short postnatal window of complete cardiac regeneration following apical resection in the neonatal mouse. Stimulated by their work we independently established a neonatal mouse model of left anterior descending artery (LAD) ligation and proved excellent recovery of murine neonatal hearts after clinical relevant myocardial infarction (MI). The underlying pathways are still poorly defined. Thus, we carried out a comprehensive analysis of the coding and non-coding transcriptome in the normal developing mouse heart and the post-MI mouse heart. These data revealed a previously unreported transition in microRNA expression in the developing heart between postnatal day three (P3) and P5 that associates specifically with cessation of cardiomyocyte cell division. To test our conclusions that these data define the major coding and non-coding transcriptional pathways for normal cardiac development and post-MI repair, we selected exemplars of miRNAs that were implicated as regulators of cardiomyocyte proliferation. Of the successfully tested miRNAs we now selected miR-6240 for further *in vitro* and *in vivo* analysis.

Using miRNA mimics the selected miRNA proved a regulatory role in cardiomyocyte proliferation in vitro. We evidenced a functional influence on mouse cardiomyocyte cell division. In addition, we show functional conservation of miR-6240 in human cardiomyocytes.

Next we established a cardiomyocyte specific miR 6240 containing AAV9 vector and applied this AAV9 in vivo. We successfully transduced cardiomyocytes of neonatal mice and studied the phenotype by means of echocardiography and histology. No significant difference was found between miR 6240 and control treated animals. These experiments are still ongoing and have not come to an end. Despite no initial overt phenotype in during physiological development we will test the influence of the miRNA during neonatal cardiac myocardial infarction and study the role during cardiac regeneration.

Publications issued by this project

So far no publications have been issued from this particular project but manuscripts are in preparation.

External funding

Investigating long non-coding RNA regulated pathways (FWF- I 4161). Amount granted: 206,892.00 €.

Miscellaneous

Our lab won a best abstract award at the annual ESC conference 2020.

Two MD students finished their diploma thesis.

Disclosure

This annual report was prepared by the Department Forschungsservice und Innovation.

Dr. María Teresa Pérez Mediavilla is responsible for the editorial part concerning the MUI-START Programme. The PIs are responsible for the content of their final reports.