DEPARTMENT OF BIOCHEMISTRY, MOLECULAR AND STRUCTURAL BIOLOGY B-1

The research activities of the members of the department are largely focused on studies of the physiological role of proteases in normal and pathological conditions, the mechanism of their action and regulation, as well as their properties and structures. Part of the activities is devoted to the development of tools that allow us to understand the properties of proteases and other enzymes, as well as to enable their monitoring and manipulation in in-vivo conditions.

Head:
Prof. Boris Turk

Protease research has undergone a major expansion in the past decade, largely due to the extremely rapid development of new technologies, such as quantitative proteomics and in-vivo imaging, as well as the extensive use of in-vivo models. These have led to the identification of physiological substrates and resulted in a paradigm shift from the concept of proteases as protein-degrading enzymes to proteases as key signalling molecules. Their catalytic activities are precisely regulated, the most important ways being zymogen activation and inhibition by their endogenous protein inhibitors. Any imbalance in this regulation can lead to pathologies such as autoimmune, neurological and cardiovascular disorders, cancer and osteoporosis. However, the molecular mechanisms of protease action are only partially understood, since only a minor subset of physiological substrates for a limited number of proteases has been identified. The role of proteases in various physiological processes is therefore still poorly understood.

One such example is the role of proteases in oxidative stress, which can be described as an increased level of reactive oxygen species (ROS) affecting a wide variety of cellular components, causing various abnormalities. For a long time, oxidative stress was associated with cell death, especially classic necrosis; however, its role in other cell-death pathways was less clear. In the article published in Biological Chemistry, we evaluated the effect of four different ROS scavengers, N-acetyl-L-cysteine (NAC), α-tocopherol (α-TOC) and two SOD mimetics, Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP) and Tempol. We focused on four different cell-death models, including menadione (MD)-triggered necrosis, staurosporine (STS)-induced apoptosis and tumour necrosis factor (TNF)-induced apoptosis or necroptosis. We observed that while the classic ROS scavenger NAC entirely prevented MD-triggered necrosis, other ROS scavengers were less efficient. Even more, ROS were found to have a marginal effect on other studied cell-death pathways. Despite that, we found that Tempol was able to substantially prevent TNF-induced apoptosis and also TNF-induced necroptosis to a lesser extent. However, the effect was likely not associated with its ROS-scavenging function, but compound-specific and the mechanism of action remains to be revealed. In our other study, we found that impaired redox homeostasis could also be the molecular mechanism behind the stefin-B mediated progression of Unverricht-Lundborg disease, which is a form of myoclonic epilepsy. Stefin B (cystatin B) is an intracellular inhibitor of cysteine cathepsins and stefin B-deficient mice were found to be more sensitive to lipopolysaccharide (LPS)-induced sepsis as a consequence of the increased expression of caspase-11 and Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing (NLRP) inflammasome activation and higher levels of mitochondrial reactive oxygen species (ROS). In our study, we investigated whether LPS-triggered oxidative stress affected the protein levels and redox status of redox sensitive proteins (thioredoxin, peroxiredoxins, and superoxide dismutases) in the macrophages and spleen of LPS-injected mice. The LPS challenge was found to result in a marked elevation in mitochondrial peroxiredoxin 3 (Prx3), sulfiredoxin, and superoxide dismutase 2 (Sod2) in stefin B-deficient macrophages and spleen. We determined that sulfiredoxin is targeted to mitochondria after the LPS challenge and that the upregulation of mitochondrial redox-sensitive proteins Prx3 and Sod2 in stefin B-deficient cells implies a protective role of stefin B in mitochondrial function. Besides studying the physiological functions of stefins, we also tried to apply them to the development of targeted drug-delivery systems. Stefins are general endogenous inhibitors of cysteine cathepsins and their specific inhibitory affinity can be utilized for targeted drug delivery in pathological conditions such as cancer, where cysteine cathepsins are abundantly overexpressed. Cathepsins S and L are also known to be secreted into the tumour micro-environment by tumour and/or immune cells, which makes them promising drug-delivery targets. We tested this hypothesis by developing a new system for cathepsin S/L, targeting using a liposomal drug-carrier system functionalized with the endogenous cysteine cathepsin inhibitor stefin A. We confirmed the selective targeting of cathepsins by stefin A-conjugated liposomes in vitro and in vivo and demonstrated the potential of this approach for cancer diagnosis and treatment.

As a continuation of our protease-specificity studies, we performed a proteomic characterization of the degradome of cysteine protease legumain, which confirmed its narrow proteolytic specificity. We have shown that legumain has a high preference for protein cleavages outside of secondary structure elements.

Besides developing our own projects, we also collaborated with research groups from Slovenia and other countries (Hungary, Croatia, Poland, Netherlands and USA), which resulted in publications in prestigious journals such as JACS, PNAS and Chemical Science. Due to our extensive expertise in proteolysis, we were invited to prepare two review papers describing the role of cysteine cathepsins on extracellular proteolysis and extracellular matrix remodelling and we also prepared an extensive review of the use of degradomics in biomarker discovery.

Some outstanding publications in the past year

1. Bratovš A, Kramer L, Mikhaylov G, Vasiljeva O, Turk B. (2019) Stefin A-functionalized liposomes as a system for cathepsins S and L-targeted drug delivery. Biochimie. Nov; 166:94-102. doi: 10.1016/j.biochi.2019.05.018.
2. Trstenjak Prebanda M, Završnik J, Turk B, Kopitar Jerala N. (2019) Upregulation of Mitochondrial Redox Sensitive Proteins in LPS-Treated Stefin B-Deficient Macrophages. Cells. Nov 21;8(12):1476. doi: 10.3390/cells8121476.
3. Vizovišek M, Fonović M, Turk B. (2019) Cysteine cathepsins in extracellular matrix remodeling: Extracellular matrix degradation and beyond. Matrix Biol. Jan;75-76:141-159. doi: 10.1016/j.matbio.2018.01.024.
4. Kavčič N, Pegan K, Vandenabeele P, Turk B. (2019) Comparative study of the differential cell death protecting effect of various ROS scavengers. Biol Chem. Jan 28;400(2):149-160. doi: 10.1515/hsz-2017-0317

Awards and Appointments

1. Andreja Bratovš: Highly Commended Poster Award, Barcelona, Spain, Perkin Elmer European In Vivo Optical Imaging User Group Meeting 2019, Stefin A-functionalized liposomes as a system for cathepsins S and L-targeted drug delivery.

Organisation of conferences, congresses and meetings

1. 36th Winter School on Proteinases and Inhibitors 2019, Tiers, Italy, 13-17 March 2019, co-organizers

INTERNATIONAL PROJECTS

1. COST CA 15203; Mitochondrial Mapping: Evolution-Age-Gender-Lifestyle-Environment
   Asst. Prof. Nataša Kopitar – Jerala
   Cost Office
2. COST CA15124; NEUBIAS - A New Network of European Bioimage Analysts to Advance Life Science Imaging
   Asst. Prof. Tina Zavašnik Bergant
   Cost Office
3. COST CA15214; An Integrative Action for Multidisciplinary Studies on Cellular Structural Networks
   Asst. Prof. Nataša Kopitar – Jerala
   Cost Office
4. The Role of Cystatins in Neuroinflamamtion
   Asst. Prof. Nataša Kopitar – Jerala
   Slovenian Research Agency
5. Cancer management with cathepsin-targeting protein-drug conjugates: application to brain tumor therapies
   Prof. Boris Turk
   Slovenian Research Agency

RESEARCH PROGRAMMES

1. Structural biology
   Prof. Dušan Turk
2. Proteolysis and its regulation
   Prof. Boris Turk

R & D GRANTS AND CONTRACTS

1. Cathepsin X inhibitors impair the resistence of tumor cells to antiprotease therapy
   Prof. Boris Turk
2. Molecular genetic biomarkers and mechanisms of unresponsiveness to biological therapyanti-TNF in patients with chronic immune diseases
   Prof. Boris Turk
3. Structural insight into the mechanism of Clostridium difficle surface formation
   Prof. Dušan Turk
4. Inhibition of Staphylococcus aureus cell wall remodeling
   Prof. Dušan Turk
5. Role of legumain in infection and inflammation
   Prof. Marko Fonović
6. Role of cysteine cathepsins in complement activation in cancer
   Prof. Boris Turk
7. Improvement of immunotherapeutic potential of NK cells through modulation of cystatin F
   Dr. Miha Butinar
8. Mineral inclusions in garnet from macroscopic to atomic scale: Opening the petrogenetic archive
   Prof. Boris Turk
9. Cathepsin-based non-invasive diagnostics and theranostics of cancer
   Prof. Boris Turk
10. Innovative ECO plasma seed treatment (for sowing and for human and animal diet/nutrition
    Prof. Boris Turk
11. Lyposomal Proteases in Semaphorin Signaling and Cell Polarity
    Prof. Boris Turk
    Icgeb - International Centre For Genetic

VISITORS FROM ABROAD

1. Prof. Kazuo Umezawa, Aichi Medical University, Nagakute, Japan, 4-6 March 2019
2. Dr Jakub Ptáček, Institute of Biotechnology CAS, Prague, Czech Republic, 9-11 July 2019
3. Michał Kanoza, Jagiellonian University, Kraków, Poland, 27 July to 30 September 2019
4. Alma Jahić, University of Tuzla, Bosnia & Herzegovina, 29 September to 11 October 2019

STAFF

Researchers
1. Dr. Iztok Dolenc
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4. Prof. Brigita Lenarčič*
5. *Abelardo Manuel Silva, B. Sc., left 15.06.19*
6. Prof. Veronika Stoka
7. Andrej Šali, B. Sc.
8. **Prof. Boris Turk, Head**
9. Prof. Dušan Turk
10. Asst. Prof. Livija Tušar
11. Prof. Olga Vasiljeva
12. Prof. Eva Žerovnik

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13. *Dr. Miha Butinar, left 01.10.19*
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15. Dr. Nežka Kavčič
16. *Dr. Jasna Lalić, left 16.10.19*
17. Dr. Nataša Lindič
18. Dr. Georgy Mikhaylov
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20. Dr. Jure Pražnikar*
21. Dr. Vida Puizdar
22. *Dr. Ajda Taler-Verčič, left 04.11.19*
23. Dr. Aleksandra Usenik
24. Dr. Robert Vidmar

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25. Monika Biasizzo, B. Sc.
26. Andreja Bratovš, B. Sc.
27. *Marija Grozdanić, B. Sc., left 01.10.19*
28. Urban Javoršek, B. Sc.
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39. Dejan Pelko
40. Polonca Pirš Kovačič

Note:
* part-time JSI member

BIBLIOGRAPHY

Original Article

1. Samra Hasanbašić, Ajda Taler-Verčič, Vida Puizdar, Veronika Stoka, Magda Tušek-Žnidarič, Andrej Vilfan, Selma Berbić, Eva Žerovnik, "Prolines affect the nucleation phase of amyloid fibrillation reaction; mutational analysis of human stefin B", *ACS chemical neuroscience*, 2019, **10**, 6, 2730-2740.
2. Urša Pečar Fonović, Milica Perišić, Nace Zidar, Brigita Lenarčič, Janko Kos, "The carboxypeptidase activity of cathepsin X is not controlled by endogenous inhibitors", *Acta chimica slovenica*, 2019, **65**, 1, 58-61.
3. Robert Vidmar, Matej Vizovišek, Dušan Turk, Boris Turk, Marko Fonović, "Characterization of legumain degradome confirms narrow cleavage specificity", *Acta chimica slovenica*, 2019, **66**, 1, 50-57.
4. Janja Božič, Iztok Dolenc, "Feedback regulation of cathepsin C by the Propeptide dipeptides of Granzymes A and B", *Acta chimica slovenica*, 2019, **66**, 2, 501-509.
5. Junjun Ni, Zhou Wu, Veronika Stoka, Jie Meng, Yoshinori Hayashi, Christoph Peters, Hong Qing, Vito Turk, Hiroshi Nakanishi, "Increased expression and altered subcellular distribution of cathepsin B in microglia induce cognitive impairment through oxidative stress and inflammatory response in mice", *Aging cell*, 2019, **18**, 1, e12856.
6. Andreja Bratovš, Lovro Kramer, Georgy Mikhaylov, Olga Vasiljeva, Boris Turk, "Stefin A-functionalized liposomes as a system for cathepsins S and L-targeted drug delivery", *Biochimie*, 2019, **199**, 94-102.
7. Katarina Korenčan, Jan Potempa, Boris Turk, "Host cell-surface proteins as substrates of gingipains, the main proteases of Porphyromonas gingivalis", *Biological chemistry*, 2019, **399**, 12, 1353-1361.
8. Nežka Kavčič, Katarina Pegan, Peter Vandenabeele, Boris Turk, "Comparative study of the differential cell death protecting effect of various ROS scavengers", *Biological chemistry*, 2019, **400**, 2, 149-160.
9. Eva Žerovnik, "Possible mechanisms by which Stefin B could regulate proteostasis and oxidative stress", *Cells*, 2019, **8**, 1, 70.
10. Mojca Trstenjak-Prebanda, Janja Završnik, Boris Turk, Nataša Kopitar-Jerala, "Upregulation of mitochondrial redox sensitive proteins in lps-treated stefin B-deficient macrophages", *Cells*, 2019, **8**, 12, 1476.
11. Ana Bajc Česnik, Simona Darovic, Sonja Prpar Mihevc, Maja Štalekar, Mirjana Malnar, Helena Motaln, Youn-Bok Lee, Julija Mazej, Jure Pohleven, Markus Grosch, Miha Modic, Marko Fonović, Boris Turk, Micha Drukker, Christopher E. Shaw, Boris Rogelj, "Nuclear RNA foci from C9ORF72 expansion mutation form paraspeckle-like bodies", *Journal of cell science*, 2019, **132**, 5, jcs224303.
12. Janja Božič, Katja Bidovec, Matej Vizovišek, Iztok Dolenc, Veronika Stoka, "Menadione-induced apoptosis in U937 cells involves Bid cleavage and stefin B degradation", *Journal of cellular biochemistry*, 2019, **120**, 6, 10662-10669.
13. Elma Mons *et al.* (11 authors), "The alkyne moiety as a latent electrophile in irreversible covalent small molecule inhibitors of cathepsin K", *Journal of the American Chemical Society*, 2019, **141**, 8, 3507-3514.
14. Tamara Marić *et al.* (12 authors), "Bioluminescent-based imaging and quantification of glucose uptake in vivo", *Nature methods*, 2019, **16**, 6, 526-532.
15. Maja Marinović Guić, Lucija Mijanović, Marko Šoštar, Matej Vizovišek, Alexander Junemann, Marko Fonović, Boris Turk, Igor Weber, Jan Faix, Vedrana Filić, "IQGAP-related protein IqgC suppresses Ras signaling during large-scale endocytosis", *Proceedings of the National Academy of Sciences of the United States of America*, 2019, **116**, 4, 1289-1298.
16. Mateja Rebernik, Brigita Lenarčič, Marko Novinec, "The catalytic domain of cathepsin C (dipeptidyl-peptidase I) alone is a fully functional endoprotease", *Protein expression and purification*, 2019, **157**, 21-27.
17. Jure Pražnikar, Miloš Tomić, Dušan Turk, "Validation and quality assessment of macromolecular structures using complex network analysis", *Scientific reports*, 2019, **9**, 1678.
18. Anastasija Panevska, Vesna Hodnik, Matej Skočaj, Maruša Novak, Špela Modic, Ivana Pavlic, Sara Podržaj, Miki Zarić, Nataša Resnik, Peter Maček, Peter Veranič, Jaka Razinger, Kristina Sepčić, "Pore-forming protein complexes from Pleurotus mushrooms kill western corn rootworm and Colorado potato beetle through targeting membrane ceramide phosphoethanolamine", *Scientific reports*, 2019, **9**, 5073.

Review Article

1. Vito Turk, Dušan Turk, Iztok Dolenc, Veronika Stoka, "Characteristics, structure, and biological role of stefins (type-1 cystatins) of human, mammal, and parasite origin", *Acta chimica slovenica*, 2019, **66**, 1, 5-17.
2. Eva Vidak, Urban Javoršek, Matej Vizovišek, Boris Turk, "Cysteine cathepsins and their extracellular roles: shaping the microenvironment", *Cells*, 2019, **8**, 3, 264.
3. Matej Vizovišek, Marko Fonović, Boris Turk, "Cysteine cathepsins in extracellular matrix remodeling: extracellular matrix degradation and beyond", *Matrix biology*, 2019, **75/76**, 141-159.

4. Marija Grozdanić, Robert Vidmar, Matej Vizovišek, Marko Fonović, "Degradomics in biomarker discovery", *Proteomics. Clinical applications.*, 2019, **13**, 6, 1800138.

Independent Component Part or a Chapter in a Monograph

1. Livija Tušar, Marjana Novič, Marjan Tušar, Jure Zupan, "Structural elucidation", In: *Encyclopedia of analytical science*, Elsevier, 2019, 278-289.