

# DEPARTMENT OF BIOTECHNOLOGY

## B-3

*At the Department of Biotechnology we investigate biological molecules of microbiological, fungal, plant and animal origin using modern biotechnological methods. We would like to apply them for diagnostic and therapeutic purposes in human and veterinary medicine, for plant protection, the preparation of high-quality and safe food and for the protection of the environment, contributing to an improvement in people's health and the environment in which we live. Our research work is focused on the processes of cancer progression and immune response, neurodegenerative processes, the biology of fungi, plant stress response and the search for new biotechnological approaches and products.*

Mushrooms are a rich source of unknown and unique proteins with exceptional properties. In 2018 we continued to identify and characterize new proteins from mushrooms. In cooperation with the Biotechnical Faculty of the University of Ljubljana, we were the first to demonstrate the presence of acetylcholinesterase activity in fungi. Acetylcholinesterases are known as one of the catalytically most efficient enzymes and play an important role in the transmission of nerve signals in vertebrates. Cholinesterases have also been described in microorganisms, protozoa, invertebrates and plants, where they mainly perform various non-neuronal regulatory roles. However, there have been no reports on cholinesterase activity in mushrooms. In our study, a strong cholinesterase activity was observed in a quarter of 45 tested mushroom aqueous extracts. A surprising variability of these enzymes was shown, and they were not characterized as vertebrate-like cholinesterases. In addition, the presence of acetylcholinesterase inhibitors was demonstrated in a fifth of aqueous extracts, with the wood pinkgill (*Entoloma rhodopolium*) extract showing an extremely potent inhibitory effect. Inhibitors of cholinesterases are used in medicine for the treatment of neuromuscular disorders and Alzheimer's disease. In addition, they are used as insecticides, pesticides and nerve agents (for example, sarin). The study confirmed the incredible potential of mushrooms as a source of unknown new natural compounds.

The search for novel antibacterial compounds from mushrooms in cooperation with the Biotechnical Faculty of the University of Ljubljana continued using the food-borne pathogenic and food spoilage bacteria and fungi. Aqueous extracts of various wild mushrooms affected the growth and adhesion of the Gram-positive bacteria *Listeria innocua* and *Bacillus cereus*, the Gram-negative bacteria *Escherichia coli* and *Campylobacter jejuni*, the yeast *Candida albicans* and the mould *Aspergillus ochraceus*.

In 2018 our studies in the field of glycobiology focused on the effect of fungal lectins on the biofilm formation of selected pathogenic bacteria. We demonstrated that lectins can either enhance or diminish the biofilm formation of different bacteria. Bacteria in biofilms are particularly resistant to antibiotics and sanitizers, as they are embedded in a self-produced matrix of extracellular material that protects them from these adverse conditions. Biofilms represent a persistent source of contamination and ensure bacterial survival through industrial food-preparation processes and in various medical settings. Lectins represent an alternative approach to the prevention of biofilm formation and can contribute to reducing the use of antibiotics. We also investigated the glycosylation profile of the inhibitor of cysteine peptidases cystatin F, which is important for the trafficking of the protein to lysosomal vesicles as well as for the internalization to the immune cells.

Cystatin F is one of the key regulators of immune cytotoxic cells. In these cells it can enter the lysosomes and cytotoxic granules and inhibits the cathepsins C and H, which are the main convertases of progranzyms, the triggers of cell death. Secretory-granule-dependent cytotoxicity is typical for natural killer (NK) cells and cytotoxic T lymphocytes, and consequently we focused our studies on these cell types. Using different cystatin F mutants we have shown that the internalization of both dimeric and monomeric forms of cystatin F leads to a reduction of the activity of cathepsins C and H in recipient cells. The internalization rate of both dimeric and monomeric cystatin F was shown to be governed by their glycosylation pattern. We have also



Head:  
**Prof. Janko Kos**

**Cysteine peptidases and their inhibitors modulate the interplay between cancer stem cells, differentiated cancer cells and immune cells in a tumour micro-environment.**



Figure 1: Acetylcholinesterase activity is present in the pear-shaped puffball (*Lycoperdon pyriforme*).

demonstrated that cystatin F can be transported to the endosomes/lysosomes of NK cells, resulting in the decreased activity of effector granzymes A and B as well as a lower cytotoxicity towards target cells.

In the previous year we extensively investigated the source of extracellular cystatin F in a tumour micro-environment. In tissue sections obtained from human brain tumours we showed, in collaboration with the National Institute of Biology, that the main source is cancer stem cells, de-differentiated cancer cells and monocytes. We proposed a model of a tumour micro-environment with the interplay between these cells and immune cytotoxic

cells. In this model cystatin F is an important mediator, causing anergy of cytotoxic cells and consequently lower cancer cell killing. On the other hand, anergic cytotoxic cells with the increased secretion of cytokines, differentiate cancer stem and monocytes to mature cells, which do not express cystatin F and are therefore unable to induce anergy. Differentiated

tumour cells are more sensitive to chemotherapeutics, which might improve the cancer treatment.

As a main transcriptional factor that regulates the expression of cystatin F, c-EBP alpha was identified. Its higher expression is associated with cystatin F in monocytes and cytotoxic cells and CEBP alpha therefore represents a key target for the regulation of cystatin F expression and the function of cytotoxic cells.

In the field of neurobiology we continued with investigations of molecular mechanisms of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) with three published articles and another two in the field of Prader Willi Syndrome and disease-associated RNA splicing. We would highlight the publication in the Brain magazine (IF 10.8) about the feedback loop between the TDP-43 protein, dipeptide repetitions, and nuclear transport in the neurodegenerative disease frontotemporal dementia. The work was carried out in collaboration with King's College London. Cytoplasmic accumulation and the aggregation of TDP-43 is the main pathological sign of ALS and FTD. In fruit-fly-based studies that model one genetic form of these diseases, it has been found that the excessive accumulation of soluble fly TDP-43 in cytoplasm causes the pathological accumulation of KPNA2 and KPNA4 proteins, important for nuclear transport, which is even more likely to prevent the transport of TDP-43 to the nucleus. A similar phenomenon was then observed in post-mortem brain patients with frontotemporal dementia. This result has identified an important feedback loop for the accumulation of TDP-43 in the cytoplasm, which could be a mechanism for the occurrence of the disease.

We continued our work on the development of new methods for the genetic engineering of lactic acid bacteria and their potential use for the delivery of therapeutic proteins to the mucosal surfaces. In the field of method development, we prepared a series of plasmid vectors that enable the concomitant controlled expression of two recombinant proteins. We were also the first to prepare a plasmid vector that enables the use of the CRISPR-Cas9 system in the lactic acid bacterium *Lactococcus lactis*. We confirmed the efficacy on several model genes that were located either in the genome or in another plasmid. Apart from that, we upgraded the CRISPR-Cas9 system into the CRISPR interference system (CRISPRi) that enables the targeted silencing of selected genes, which is useful in the regulation of signal pathways.

In the field of the development of therapeutic delivery systems, we collaborated with a Czech group (Dr. P. Mały, BIOCEV, Vestec) to develop binders of the p19 subunit of human pro-inflammatory cytokine IL-23 that plays an important role in inflammatory bowel disease and psoriasis. Binders were displayed on the surface of *L. lactis* and their ability to remove IL-23 from the solution was confirmed. This activity could be useful in decreasing inflammation and we would like to confirm it in future studies in an appropriate animal model.

We have also developed a system for the delivery of the therapeutic peptide BPC-157, whereby we compared the delivery by secretion from bacterial cells, and the delivery by surface display followed by a controlled release mediated by the digestive protease trypsin. BPC-157 is a gastric-stable pentadecapeptide that prevents and treats inflammations of the gastrointestinal tract via its anti-oxidative action. By using custom-developed antibodies and HPLC, we confirmed that a larger quantity of BPC-157 is delivered by using cell secretion from *L. lactis*. The effective delivery of the peptide was confirmed in a fibroblast cell model, where a statistically significant

**The CRISPR/Cas9 system for gene editing was adopted for use in lactic acid bacterium *Lactococcus lactis*.**

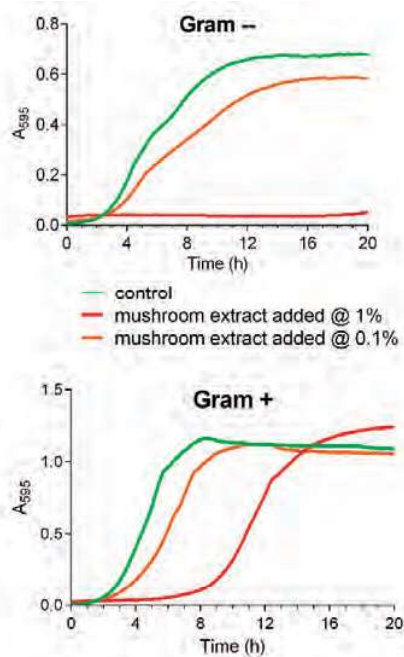


Figure 2: Growth curve. Mushroom extract negatively affects the growth of Gram-negative and Gram-positive bacteria at a very low concentration.

**An important feedback loop for the accumulation of TDP-43 in the cytoplasm in patients with frontotemporal dementia has been identified, explaining the mechanism for the occurrence of the disease.**

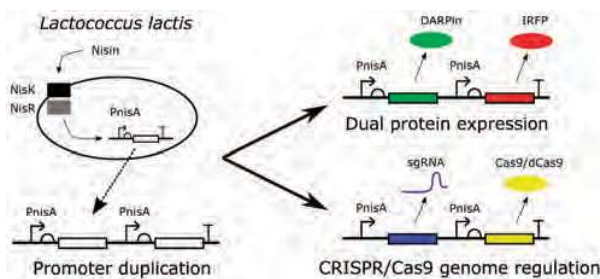


Figure 3. Duplication of the nisin promoter enables the controlled expression of two model proteins (DARPin, IRFP) in *L. lactis*, while modification of these systems enables the controlled expression/transcription of elements of CRISPR/Cas9 system (Cas9, sgRNA).

decrease in the concentration of reactive oxygen species, important players in inflammation, was observed

The results of the research work at the Department of Biotechnology in 2018 were published in 37 scientific papers in journals with an impact factor. We received three research grants from the Slovenian Research Agency. Dr. Aleš Berlec received the Lapanje plaque award from the Slovenian Biochemical Society for excellent professional achievements. The members of the department were also very active in pedagogical work as lecturers and mentors to students preparing diploma and doctoral thesis at universities in Slovenia and abroad. In 2018 two doctoral theses were completed at the department.

### Some outstanding publications in the past year

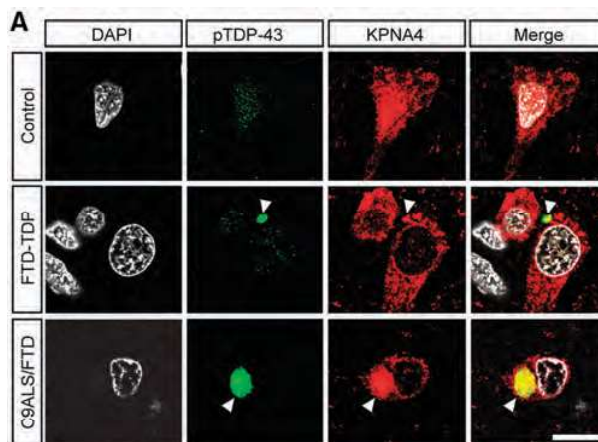
1. Solomon, Daniel A., Rogelj, Boris, et al. A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin-[alpha] mediates C9orf72-related neurodegeneration. *Brain : journal of neurology*, ISSN 0006-8950, [in press] 2018, IF 10,84.
2. Pišlar, Anja, Jewett, Anahid, Kos, Janko. Cysteine cathepsins : their biological and molecular significance in cancer stem cells. *Seminars in cancer biology*, ISSN 1044-579X. [Print ed.], 2018, IF 10,12.
3. Kaur, Kawaljit, Perišić, Milica, Ko, Meng-Wei, Safaie, Tahgineh, Kos, Janko, Jewett, Anahid. Natural killer cells target and differentiate cancer stem-like cells/undifferentiated tumors : strategies to optimize their growth and expansion for effective cancer immunotherapy. *Current opinion in immunology*, ISSN 0952-7915. [Print ed.], 2018, vol. 51, str. 170-180, IF 7,93.

### Awards and Appointments

1. Aleš Berlec: Lapanje plaque, Ljubljana. Awarded by the Slovenian Biochemical Society for expert and organizational work in society.

### Organization of conferences and meetings

1. Annual meeting of co-workers of the research programme Pharmaceutical Biotechnology: Knowledge for Health, from the Department of Biotechnology at the Jožef Stefan Institute and the Chair of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Ljubljana, 22. 11. 2018



**Figure 4.** Frontotemporal dementia associated mislocalization of TDP-43 and KPNA4. In the postmortem brain of FTD patients, the formation of aggregates TDP-43 (green) is noticeable. Aggregates are formed outside the nucleus (white) and are associated with a modified localization of the nuclear transporter KPNA4 (red). (Solomon et al., *Brain* 2018)

## INTERNATIONAL PROJECTS

1. Pathological Mechanisms of TDP-43 in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia  
Prof. Boris Rogelj  
Slovenian Research Agency
2. Regulation of Cytotoxicity of „Super Charged“ Natural Killer Cells with Cystatin F  
Prof. Janko Kos  
Slovenian Research Agency
3. ALS and FTD Relevant Characterization of In Vivo Protein Interactors of FUS  
Prof. Boris Rogelj  
Slovenian Research Agency

## RESEARCH PROGRAM

1. Pharmaceutical Biotechnology: Knowledge for Health  
Prof. Janko Kos

## R & D GRANTS AND CONTRACTS

1. Evaluation of possible harmful effects of nanoparticles and underlying mechanisms -

from physico-chemical and in vitro toxicity characterisation to innate immune system activation

- Prof. Boris Rogelj
2. Nuclear transport defect in neurodegenerative diseases  
Prof. Boris Rogelj
3. Cathepsin X inhibitors impair the resistance of tumor cells to antiprotease therapy  
Prof. Janko Kos
4. Pathogenic role of paraspeckle-like nuclear bodies in neurodegenerative diseases ALS and FTD  
Prof. Boris Rogelj
5. Inhibition of cathepsin X activity as a novel strategy for the treatment of Parkinson's disease  
Prof. Janko Kos
6. Targeting Campylobacter adhesion in the fight against antimicrobial resistance  
Dr. Jerica Sabotič
7. Targeting, imaging and treating of colorectal cancer with safe theranostic bacteria  
Asst. Prof. Aleš Berlec
8. Phase transitions in systems of nucleotide repeat expansions associated with neurodegenerative diseases  
Prof. Boris Rogelj
9. Lactic Acid Bacteria-Mediated Intestinal Delivery of Novel Therapeutic Protein Binders Derived from Scaffold of Albumin-Binding Domain  
Asst. Prof. Aleš Berlec  
ZRC SAZU

## VISITORS FROM ABROAD

1. Jakub Nowak, Nanotemper Technologies GmbH, Munich, Germany, 1 March 2018
2. Lior Levy, M. Sc., Faculty of Biotechnology and Food Engineering, Israel Institute of Technology, Haifa, Israel, 10 June – 15 June 2018
3. Dr. Peter Malý, Institute of Biotechnology of the Czech Academy of Science, Prague, Czech Republic, 13 September – 18 September 2018

## STAFF

### Researchers

1. Asst. Prof. Aleš Berlec
2. Prof. Janko Kos\*, Head
3. Asst. Prof. Helena Motaln
4. Prof. Boris Rogelj
5. Dr. Jerica Sabotič
6. Prof. Borut Štrukelj\*

### Postdoctoral associates

7. Dr. Janja Božič
8. Dr. Ana Mitrović
9. Dr. Milica Perišić Nanut
10. Dr. Anja Pucer Janež

### Postgraduates

11. Ana Bajc Česnik, B. Sc.
12. Mirjana Malnar, B. Sc.
13. Tina Vida Plavec, B. Sc.
14. Mateja Prunk, B. Sc.
15. Emanuela Senjor, B. Sc.
16. *Katja Škrlec, B. Sc., left 01.11.18*

### Technical and administrative staff

17. Maja Šimaga, M. Sc.

### Note:

\* part-time JSI member

## BIBLIOGRAPHY

### ORIGINAL ARTICLE

1. Dawid Panek, Anna Więckowska, Jakub Jończyk, Justyna Godyń, Marek Bajda, Tomasz Wichur, Anna Pasięka, Damijan Knez, Anja Pišlar, Jan Korabecny, Ondrej Soukup, Vendula Sepsova, Raimon Sabaté, Janko Kos, Stanislav Gobec, Barbara Malawska, "Design, synthesis and biological evaluation of 1-benzylamino-2-hydroxyalkyl derivatives as new potential disease-modifying multifunctional anti-Alzheimer's agents", *ACS chemical neuroscience*, 2018, **9**, 5, 1074-1094.
2. Katja Škrlec, Rudolf Ručman, Eva Jarc, Predrag Sikirić, Urban Švajger, Toni Petan, Milica Perišić, Borut Štrukelj, Aleš Berlec, "Engineering recombinant *Lactococcus lactis* as a delivery vehicle for BPC-157 peptide with antioxidant activities", *Applied microbiology and biotechnology*, 2018, **102**, 23, 10103-10117.
3. Izidor Sosič, Ana Mitrović, Hrvoje Čurić, Damijan Knez, Helena Brodnik Žugelj, Bogdan Štefane, Janko Kos, Stanislav Gobec, "Cathepsin B inhibitors: further exploration of the nitroxoline core", *Bioorganic & Medicinal Chemistry Letters*, 2018, **28**, 7, 1239-1247.
4. Daniel A. Solomon *et al.* (25 authors), "A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin- $\alpha$  mediates C9orf72-related neurodegeneration", *Brain: journal of neurology*, 2018, **141**, 10, 2908-2924.
5. Gordana Glavan, Monika Kos, Janko Božič, Damjana Drobne, Jerica Sabotič, Anita Jemec Kokalj, "Different response of acetylcholinesterases in salt- and detergent-soluble fractions of honeybee haemolymph, head and thorax after exposure to diazinon", *Comparative biochemistry and physiology. Part C, Toxicology & pharmacology*, 2018, **205**, 8-14.
6. Janko Ignjatovic, Urban Švajger, Matjaž Ravnikar, Peter Molek, Darko Zadavec, Alenka Pariš, Borut Štrukelj, "Aggregation of recombinant monoclonal antibodies and its role in potential immunogenicity", *Current pharmaceutical biotechnology*, 2018, **19**, 4, 343-356.
7. Esmeralda Dautović, Milica Perišić, Adaleta Softić, Janko Kos, "The transcription factor C/EBP  $\alpha$  controls the role of cystatin F during the differentiation of monocytes to macrophages", *European journal of cell biology*, 2018, **97**, 7, 463-473.
8. Damijan Knez, Nicolas Coquelle, Anja Pišlar, Simon Žakelj, Marko Jukič, Matej Sova, Janez Mravljak, Florian Nachon, Xavier Brazzolotto, Janko Kos, Jacques-Philippe Colletier, Stanislav Gobec, "Multi-target-directed ligands for treating Alzheimer's disease: butyrylcholinesterase inhibitors displaying antioxidant and neuroprotective activities", *European Journal of Medicinal Chemistry*, 2018, **156**, 598-617.
9. Péter Ábrányi-Balogh, László Petri, Tímea Imre, Péter Szijj, Andrea Scarpino, Martina Hrast, Ana Mitrović, Urša Pečar Fonović, Kristina Németh, Hélène Barreateau, David I. Roper, Kata Horváti, György G. Ferenczy, Janko Kos, Janez Ilaš, Stanislav Gobec, György Keserü M., "A road map for prioritizing warheads for cysteine targeting covalent inhibitors", *European Journal of Medicinal Chemistry*, 2018, **160**, 94-107.
10. Anja Pišlar, Larisa Tratnjek, Gordana Glavan, Marko Živin, Janko Kos, "Upregulation of cysteine protease cathepsin X in the 6-hydroxydopamine model of Parkinson's disease", *Frontiers in molecular neuroscience*, Nov. 2018, **11**, 412.
11. Katarina Vrabec, Emanuela Boštjančič, Blaž Koritnik, Lea Leonardis, Leja Dolenc-Grošelj, Janez Zidar, Boris Rogelj, Damjan Glavač, Metka Ravnik-Glavač, "Differential expression of several miRNAs and the host genes AATK and DNM2 in leukocytes of sporadic ALS patients", *Frontiers in molecular neuroscience*, 2018, **11**, 106.
12. I. Donadon *et al.* (11 authors), "Exon Specific U1 snRNAs improve ELP1 exon 20 definition and rescue ELP1 protein expression in a Familial Dysautonomia mouse model", *Human molecular genetics*, 2018, **27**, 14, 2466-2476.
13. Katja Škrlec, Petra Zadavec, Marie Hlavničková, Milan Kuchař, Lucie Vaňková, Hana Petroková, Lucie Křížová, Jiří Černý, Aleš Berlec, Petr Malý, "p19-targeting ILP protein blockers of IL-23/Th-17 pro-inflammatory axis displayed on engineered bacteria of food origin", *International journal of molecular sciences*, 2018, **19**, 7, 1933.
14. Nevena Maljurić, Jelena Golubović, Matjaž Ravnikar, Dušan Žigon, Borut Štrukelj, Biljana Otašević, "Isolation and determination of fomentariol: Novel Potential antidiabetic drug from fungal material", *Journal of Analytical Methods in Chemistry*, 2018, 2434691.
15. Mojca Božič, Vinko Boc, Urša Pečar Fonović, Janja Marc, Aleš Blinc, Janko Kos, Darko Černe, "Increased plasma cathepsin S at the time of percutaneous transluminal angioplasty is associated with 6-months' restenosis of the femoropopliteal artery", *Journal of Medical Biochemistry*, 2018, **37**, 1, 54-61.
16. Urban Košak, Boris Brus, Damijan Knez, Simon Žakelj, Jurij Trontelj, Anja Pišlar, Roman Šink, Marko Jukič, Marko Živin, Adrian Podkowa, Florian Nachon, Xavier Brazzolotto, Jure Stojan, Janko Kos, Nicolas Coquelle, Kinga Sałat, Jacques-Philippe Colletier, Stanislav Gobec, "The magic of crystal structure-based Inhibitor optimization: development of a butyrylcholinesterase inhibitor with picomolar affinity and In vivo activity", *Journal of medicinal chemistry*, 2018, **61**, 1, 119-139.
17. Barbara Breznik, Clara Limbäck-Stokin, Janko Kos, Mohammed Khurshed, Vashendriya V. V. Hira, Roman Bošnjak, Tamara Lah Turnšek, Cornelis J. F. van Noorden, "Cysteine cathepsins B, X and K expression in peri-arteriolar glioblastoma stem cell niches", *Journal of molecular histology*, 2018, **49**, 5, 481-497.
18. Mojca Lunder, Miha Vodnik, Valentina Kubale, Neža Grgurevič, Gregor Majdič, Borut Štrukelj, "Peptide mimetic of N-terminal ghrelin enhances ghrelin induced growth hormone secretion and c-Fos expression in mice", *Journal of neuroendocrinology*, 2018, **30**, 12, e12656.

19. Camila Bonturi *et al.* (11 authors), "Could a plant derived protein potentiate the anticancer effects of a stem cell in brain cancer?", *Oncotarget*, 2018, **9**, 30, 21296-21312.
20. Mateja Starbek Zorko, Borut Štrukelj, Urban Švajger, Samo Kreft, Tomaž Lunder, "Efficacy of a polyphenolic extract from silver fir (*Abies alba*) bark on psoriasis: a randomised, double-blind, placebo-controlled trial", *Pharmazie*, 2018, **73**, 1, 56-60.
21. Mojca Lunder, Irena Roškar, Jan Hošek, Borut Štrukelj, "Silver fir (*Abies alba*) extracts inhibit enzymes involved in blood glucose management and protect against oxidative stress in high glucose environment", *Plant foods for human nutrition*, 2018, **74**, 1, 47-53.
22. Alenka Vesel, Nina Recek, Helena Motaln, Miran Mozetič, "Endothelialization of polyethylene terephthalate treated in SO<sub>2</sub> plasma determined by the degree of material cytotoxicity", *Plasma*, 2018, **1**, 1-11.
23. Janja Božič, Veronika Stoka, Iztok Dolenc, "Glucosamine prevents polarization of cytotoxic granules in NK-92 cells by disturbing FOXO1/ERK/paxillin phosphorylation", *PLoS one*, 2018, **13**, 7, 0200757.
24. Barbara Breznik, Clara Limback, Andrej Porčnik, Andrej Blejec, Miha Koprivnikar Krajnc, Roman Bošnjak, Janko Kos, Cornelis J. F. van Noorden, Tamara Lah Turnšek, "Localization patterns of cathepsins K and X and their predictive value in glioblastoma", *Radiology and oncology*, 2018, **52**, 4, 433-442.
25. Tomaž Bratkovič, Miha Modic, Camargo Camargo Ortega, Micha Drukker, Boris Rogelj, "Neuronal differentiation induces SNORD115 expression and is accompanied by post-transcriptional changes of serotonin receptor 2c mRNA", *Scientific reports*, 2018, **8**, 5101.
26. Anja Kovanda, Lea Leonardis, Janez Zidar, Blaž Koritnik, Leja Dolenc-Grošelj, Stanislava Ristić Kovačič, Tomaž Curk, Boris Rogelj, "Differential expression of microRNAs and other small RNAs in muscle tissue of patients with ALS and healthy age-matched controls", *Scientific reports*, 2018, **8**, 5609.
27. Aleš Berlec, Katja Škrlec, Janja Kocjan, Maria Olenic, Borut Štrukelj, "Single plasmid systems for inducible dual protein expression and for CRISPR-Cas9/CRISPRi gene regulation in lactic acid bacterium *Lactococcus lactis*", *Scientific reports*, 2018, **8**, 1009.
28. Mona das Neves Oliveira, Micheli M. Pillat, Helena Motaln, Henning Ulrich, Tamara Lah Turnšek, "Kinin-B1 receptor stimulation promotes invasion and is involved in cell-cell interaction of co-cultured glioblastoma and mesenchymal stem cells", *Scientific reports*, 2018, **8**, 1299.
29. Anja Pišlar, Anahid Jewett, Janko Kos, "Cysteine cathepsins: their biological and molecular significance in cancer stem cells", *Seminars in cancer biology*, Dec. 2018, **53**, 168-177.
30. Anahid Jewett, Janko Kos, Yuman Fong, Meng-Wei Ko, Tahmineh Safaei, Milica Perišić, Kawaljit Kaur, "NK cells shape pancreatic and oral tumor microenvironments; role in inhibition of tumor growth and metastasis", *Seminars in cancer biology*, 2018, **53**, 178-188.

## REVIEW ARTICLE

1. Janko Kos, Milica Perišić, Mateja Prunk, Jerica Sabotič, Esmeralda Dautović, Anahid Jewett, "Cystatin F as a regulator of immune cell cytotoxicity", *Cancer immunology and immunotherapy*, 2018, **67**, 12, 1931-1938.
2. Kawaljit Kaur, Milica Perišić, Meng-Wei Ko, Tahgineh Safaie, Janko Kos, Anahid Jewett, "Natural killer cells target and differentiate cancer stem-like cells/undifferentiated tumors: strategies to optimize their growth and expansion for effective cancer immunotherapy", *Current opinion in immunology*, 2018, **51**, 170-180.
3. Aleš Berlec, "Importance of probiotics in infections", *Farmaceutski vestnik: strokovno glasilo slovenske farmacije*, 2018, **69**, 2, 148-152.

## MENTORING

1. Staša Kosler, *Development of genetically modified lactic acid bacteria for the treatment of inflammatory bowel disease: doctoral dissertation*, Ljubljana, 2018 (mentor Borut Štrukelj; co-mentor Aleš Berlec).
2. Mateja Starbek Zorko, *Inhibition of expression of proinflammatory cytokines from keratinocytes with antisense oligonucleotides and polyphenolic extract from fir bark (*Abies alba*), and its influence on mild psoriasis: doctoral dissertation*, Ljubljana, 2018 (mentor Tomaž Lunder; co-mentor Borut Štrukelj).
3. Katja Škrlec, *Surface display of evasins and bepecin on the bacteria *Lactococcus lactis* NZ9000 and *Lactobacillus salivarius* ATCC 11741 and evaluation of their anti-inflammatory action: doctoral dissertation*, Ljubljana, 2018 (mentor Aleš Berlec).