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, preparation of quality and safe food and for the protection of the environment, contributing to an improvement in peoples' 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 in the search for new biotechnological approaches and products.

Head:
Prof. Janko Kos

Mushrooms represent a promising source of bioactive proteins and biopesticides based on their own arsenal of various compounds used against pests, parasites and pathogens. Among them the defence proteins are very important and include lectins and protease inhibitors with strong and specific insecticidal and/or nematicidal activity. Phylogenetic analyses have revealed that these proteins are widely present among higher fungi, ascomycetes and basidiomycetes, which means that these protein toxins have potential applications in veterinary and human medicine, biotechnology and in crop protection.

For lectin MpL we demonstrated that it represents an effective tool to deliver therapeutic and other proteins to the tumour cells. Proteins, which by themselves are not able to enter the cells, can reach their intracellular targets by support of this lectin. The results have been published in the prestigious journal Oncotarget. Additionally, we confirmed the exclusive cytotoxicity of CNL lectin from clouded funnel against leukemic T cells Jurkat causing programmed cell death through binding a receptor in a plasma membrane. In several other papers we published the properties of other compounds of mushroom origin and their applicability in crop protection or their cytotoxic activity.

Cystatin F regulates the cytotoxicity of natural killer cells.

Investigating the role of proteolytic enzymes in the regulation of cell cytotoxicity we focused our research work on cathepsins C and H, two main convertases of progranzyme B, the molecule that triggers the processes of cell death. The activity of cathepsins C and H in secretory granules is controlled by the endogenous inhibitor cystatin F.

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 the dimeric and monomeric forms of cystatin F leads to a reduction in 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. Finally, we have shown that both dimeric and monomeric cystatin F can be transported to endosomes/lysosomes of NK cells resulting in the decreased activity of the effector granzymes A and B as well as lower cytotoxicity towards target cells. The source of extracellular cystatin F could be tumour cells, which by secreting of this inhibitor inactivate an anti-tumour immune response. These findings identify cystatin F as one of the possible mediators of tumour-induced immunosuppression and a possible therapeutic target. By lowering the uptake of extracellular cystatin F and/or its activation the efficacy of NK cell cytotoxicity could be increased in order to improve the immunotherapy of cancer patients. These results were published in Frontiers in Immunology.

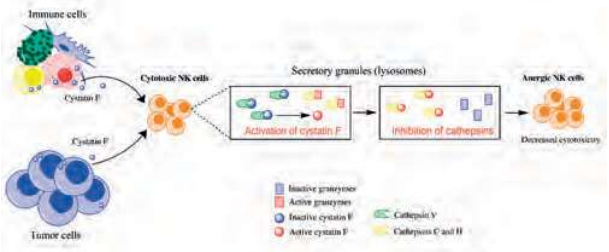

Figure 1: Schematic representation of the effects of increased extracellular concentration of cystatin F on natural killer (NK) cells.

We have also examined the regulation of cystatin F expression in immune cells and identified the transcription factor CEBP alpha as one of the regulators of cystatin F expression.

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

In the field of neurobiology we continued investigations of molecular mechanisms of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). We published an article on the use of neuromuscular co-culture in the investigation of intracellular localization of FUS and TDP-43 proteins (Journal of Molecular Neuroscience). We also reported the effect of nanoparticles on the intracellular localization of these two proteins (Neurotoxicity Research). In collaboration with the group at King's College London, we published

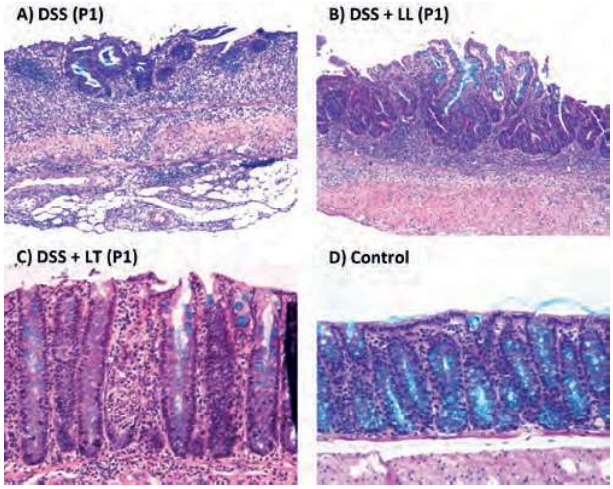

Figure 2: Representative micrographs of mice colonic mucosa from each group using Kreyberg-Jareg staining. A) Transmural inflammation accompanied by small and shallow erosion one week after DSS-induced colitis. B) Moderate inflammation limited to mucosa and submucosa one week after DSS induced colitis treated with control L. lactis. C) Moderate inflammation limited to mucosa and submucosa one week after DSS induced colitis treated with control TNFα-binding L. lactis. Note increased height of crypts and crypt abscess. D) Healthy colon mucosa. Magnifications: A - 100x; B - 100x; C - 200x; D - 200x.

The toxic effect of dipeptide repeats resulting from the mutation of hexanucleotide repeats in the C9orf72 gene is associated with amyotrophic lateral sclerosis.

A member of the department, Dr. Boris Rogelj, received a Lapanje award, the highest acknowledgement from the Slovenian Biochemical Society for excellent research achievements.

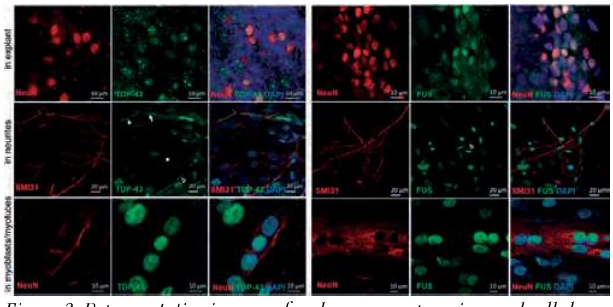

Figure 3: Representative images of endogenous expression and cellular localisation of TDP-43 and FUS in an in vitro model of innervated human muscle. Occasional cytoplasmic TDP-43 aggregates were seen in neuronal somas (arrow) and in neurites (filled arrow heads). In neurites, uneven cytoplasmic TDP-43 and FUS expression was also noted (empty arrow heads). In the muscle cells, TDP-43 and FUS were localised in the nuclei of the myoblasts and myotubes. NeuN labelled the neuronal nuclei, and SMI31 labelled the neurons. Scale bars, as given for individual panels. The nuclei were stained by DAPI (blue) (Prpar Mihevc et al., Journal of Molecular Neuroscience 2017).

a study on the toxic effect of dipeptide repeats resulting from the mutation of hexanucleotide repeats in the C9orf72 gene (Human Molecular Genetics). We have established that the toxic effect of dipeptide repetition of GA can be reduced by dipeptide repetition of PA. We also published two review articles in prestigious journals (Brain, Progress in Neurobiology).

Inflammatory bowel disease is a chronic inflammation of the gastrointestinal tract that can manifest itself as Crohn's disease or ulcerative colitis. Neutralization of pro-inflammatory cytokines with monoclonal antibodies represents an established treatment approach; however, it is associated with high costs and side effects. At the department we have developed lactic acid bacteria that are capable of neutralizing pro-inflammatory cytokines and chemokines and enable local delivery to mucosal surfaces.

We have displayed on the surface of lactic acid bacterium *Lactococcus lactis* tick proteins evasins that are capable of binding different chemokines. By using recombinant bacteria we were able to remove 15–90 % of eleven chemokines. The ability to remove chemokine CXCL-8 and decrease its secretion was confirmed in a Caco-2 cellular model of the epithelium. We have also coated the lactic acid bacterium *Lactobacillus salivarius* with binders of cytokines IL-17, IL-23 in TNFα by using a previously developed approach of non-GMO surface display. We were the first to prepare the bacteria with the ability to concomitantly bind three different cytokines. The bacteria were assessed by using different concentrations of bacteria and different concentrations of cytokines. Their stability in simulated gastric juice was confirmed. Bacterium *Lactococcus lactis* with TNFα-binding ability was evaluated in a mouse model of colitis, induced with sodium dextran sulphate. The decrease of the symptoms of colitis and improvement of histological picture of inflammation was effectively achieved with wild-type *Lactococcus lactis* bacteria, especially when they were administered in a preventive manner. Surprisingly, the TNFα-binding bacteria were shown to be less efficient. This is probably due to the properties of a specific animal model, for which it was previously shown that the neutralization of TNFα is not necessarily beneficial. This finding was additionally confirmed by administering a specific monoclonal antibody that has worsened the symptoms of colitis. TNFα-binding bacteria and monoclonal antibody have also increased the tissue concentration of TNFα in healthy mice to a similar level, serving as additional proof of similar activity, as well as the efficacy of developed bacteria.

The results of the research work at the Department of Biotechnology in 2017 were published in 26 scientific papers in journals with an impact factor. Also, one national and one international patent were granted and one patent application was filed. We received two research grants from the Slovenian Research Agency, one bilateral international grant and international research grant WADA. Dr. Boris Rogelj received the Lapanje award, the highest acknowledgement from the Slovenian Biochemical Society for excellent research achievements. Dr. Ana Mitrović received a Krka award for her research work. 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.

Some outstanding publications in the past year

1. Perišić, Milica, Sabotič, Jerica, Švajger, Urban, Jewett, Anahid, Kos, Janko. Cystatin F affects natural killer cell cytotoxicity. Frontiers in immunology, ISSN 1664-3224, 2017, vol. 8, pages 1459-1-1459-14, doi: 10.3389/fimmu.2017.01459. [COBISS.SI-ID 30930471].
2. Prpar Mihevc, Sonja, Darovic, Simona, Kovanda, Anja, Bajc Česnik, Ana, Župunski, Vera, Rogelj, Boris. Nuclear trafficking in amyotrophic lateral sclerosis and frontotemporal lobar degenera-

tion. Brain : journal of neurology, ISSN 0006-8950, 2017, vol. 140, issue 1, doi: 10.1093/brain/aww197, [COBISS.SI-ID 29663527] .

3. Žurga, Simon, Perišić, Milica, Kos, Janko, Sabotič, Jerica. Fungal lectin MpL enables entry of protein drugs into cancer cells and their subcellular targeting. Oncotarget, ISSN 1949-2553, 2017, doi: 10.18632/oncotarget.15849, [COBISS.SI-ID 30318887].

Awards and Appointments

1. Polona Megušar: Biotechnical Faculty Prešeren Prize 2017, Biotechnical Faculty, University of Ljubljana, Ljubljana, Characterisation of antimicrobial and antiadhesive activity of mushrooms aqueous extracts, 21 December 2017
2. Ana Mitrović: Krka Prize, Novo mesto, Slovenia, Krka, Role of cysteine cathepsins B and X and their inhibitors in epithelial-mesenchymal transition of tumour cells, 13 October 2017
3. Boris Rogelj: Lapanje Award, Bled, Slovenian Biochemical Society, for exceptional achievements in biochemical science, 21 September 2017

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, 23 November 2017
2. 8th Conference on Experimental and Translational Oncology, Association of Radiology and Oncology, Portorož, 19 April – 23 April 2017 (co-organizers)

Patents granted

1. Mojca Lunder, Matjaž Ravnikar, Borut Štrukelj, Aleš Berlec, Boris Čeh, Modified food grade microorganism for treatment of inflammatory bowel disease, EP2521737 (B1), European Patent Office, 30. 08. 2017.
2. Katja Žmitek, Nataša Tavčar, Tina Pogačnik, Janko Žmitek, Petra Keršmanc, Tadej Rejc, Uroš Petrič, Borut Štrukelj, Samo Kreft, Extract from the wood of trees of the genus fir to prevent, alleviate or treat unwanted skin changes and preparations, SI25053 (A), Urad RS za intelektualno lastnino, 31. 03. 2017.

INTERNATIONAL PROJECTS

1. Functional and Structural Studies of Lectins from Mushrooms
   Dr. Jerica Sabotič
   Slovenian Research Agency
2. The Role of C/EBP Alpha in Regulation of Cystatin F Expression
   Prof. Janko Kos
   Slovenian Research Agency
3. Pathological Mechanisms of TDP-43 in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia
   Prof. Boris Rogelj
   Slovenian Research Agency

RESEARCH PROGRAM

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

R & D GRANTS AND CONTRACTS

1. Genetics and pharmacogenomics of inflammatory bowel diseases and genetically related chronic immune diseases
   Prof. Boris Rogelj
2. Pathogenic mechanism of the C9orf72 expanded hexanucleotide repeat mutation in neurodegeneration
   Prof. Boris Rogelj
3. 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
4. Nucleart transport defect in neurodegenerative diseases
   Prof. Boris Rogelj
5. Cathepsin X inhibitors impair the resistence of tumor cells to antiprotease therapy
   Prof. Janko Kos
6. The role of cysteine protease inhibitors in NK cell mediated lysis of tumour cells
   Prof. Janko Kos

VISITORS FROM ABROAD

1. Esmeralda Dautović, M. Sc., Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina, 27 February– 22 April 2017
2. Prof. Anahid Jewett, University of California, Los Angeles, USA, 10 April 2017
3. Dr. Annabelle Varrot, Centre de Recherches sur les Macromolécules Végétales, CERMAV, Grenoble, France, 9 May – 24 May 2017
4. Aurore Cabanettes, Centre de Recherches sur les Macromolécules Végétales, CERMAV, Grenoble, France, 9 May – 24 May 2017
5. Katarzyna Walkiewicz, Nanotemper Technologies GmbH, München, Germany, 24 May 2017
6. Maria Hlavnickova, Institute of Biotechnology, The Czech Academy of Sciences, Prague, Czech Republic, 28 May – 9 June 2017
7. Dr. Peter Malý, Institute of Biotechnology, The Czech Academy of Sciences, Prague, Czech Republic, 6 July 2017
8. Esmeralda Dautović, M. Sc., Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina, 6 August – 26 August 2017
9. Prof. dr. Serge Pérez, Université Grenoble Alpes, Grenoble, France, 17 October – 18 October 2017
10. Dr. Annabelle Varrot, Centre de Recherches sur les Macromolécules Végétales, CERMAV, Grenoble, France, 17 October – 18 October 2017

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. Vida Kocbek, left 16.01.17*
8. *Dr. Anja Kovanda, left 01.07.17*
9. Dr. Ana Mitrović
10. Dr. Milica Perišić Nanut
11. *Dr. Sonja Prpar Mihevc, left 01.05.17*
12. Dr. Anja Pucer Janež

Postgraduates
13. Ana Bajc Česnik, B. Sc.
14. Mirjana Malnar, B. Sc.
15. Mateja Prunk, B. Sc.
16. Katja Škrlec, B. Sc.

Technical and administrative staff
17. Maja Šimaga, M. Sc.

Note:

* part-time JSI member

BIBLIOGRAPHY

Original Article

1. Aleš Berlec, Martina Perše, Matjaž Ravnikar, Mojca Lunder, Andreja Erman, Anton Cerar, Borut Štrukelj, "Dextran sulphate sodium colitis in C57BL/6J mice is alleviated by Lactococcus lactis and worsened by the neutralization of tumor necrosis Factor *α*", *International immunopharmacology*, **43**, 219-226, 2017.
2. Barbara Breznik, Helena Motaln, Miloš Vittori, Ana Rotter, Tamara Lah Turnšek, "Mesenchymal stem cells differentially affect the invasion of distinct glioblastoma cell lines", *Oncotarget*, **8**, 15, 25482-25499, 2017.
3. Anja Klančnik, Polona Megušar, Meta Sterniša, Barbara Jeršek, Franz Bucar, Sonja Smole Možina, Janko Kos, Jerica Sabotič, "Aqueous extracts of wild mushrooms show antimicrobial and antiadhesion activities against bacteria and fungi", *PTR, Phytother. res.*, **31**, 1971-1976, 2017.
4. Staša Kosler, Borut Štrukelj, Aleš Berlec, "Lactic acid bacteria with concomitant IL-17, IL-23 and TNF[alpha]- binding ability for the treatment of inflammatory bowel disease", *Current pharmaceutical biotechnology*, **18**, 4, 318-326, 2017.
5. Urban Košak, Damijan Knez, Nicolas Coquelle, Boris Brus, Anja Pišlar, Florian Nachon, Xavier Brazzolotto, Janko Kos, Jacques-Philippe Colletier, Stanislav Gobec, "N-propargylpiperidines with naphthalene-2-carboxamide or naphthalene-2- sulfonamide moieties", *Bioorg. med. chem.*, **25**, 2, 633-645, 2017.
6. Youn-Bok Lee *et al.* (15 authors), "C9orf72 poly GA RAN-translated protein plays a key role in amyotrophic lateral sclerosis via aggregation and toxicity", *Hum Mol Genet*, **26**, 24, 4765-4777, 2017.
7. Jasna Lojk, Sonja Prpar Mihevc, Vladimir Boštjan Bregar, Mojca Pavlin, Boris Rogelj, "The effect of different types of nanoparticles on FUS and TDP-43 solubility and subcellular localization", *Neurotox. res.*, **32**, 3, 325-339, Oct. 2017.
8. Tjaša Lukanc, Jože Brzin, Janko Kos, Jerica Sabotič, "Trypsin-specific inhibitors from the Macrolepiota procera, Armillaria mellea and Amanita phalloides wild mushrooms", *Acta Biochim. Pol.*, **64**, 1, 21-24, 2017.
9. Ana Mitrović, Urša Pečar Fonović, Janko Kos, "Cysteine cathepsins B and X promote epithelial-mesenchymal transition of tumor cells", *Eur. j. cell biol.*, **96**, 6, 622-631, 2017.
10. Ana Mitrović, Izidor Sosič, Špela Kos, Urša Lampreht Tratar, Barbara Breznik, Simona Kranjc, Bojana Mirković, Stanislav Gobec, Tamara Lah Turnšek, Maja Čemažar, Gregor Serša, Janko Kos, "Addition of 2-(ethylamino)acetonitrile group to nitroxoline results in significantly improved anti-tumor activity in vitro and in vivo", *Oncotarget*, **8**, 35, 59136-59147, 2017.
11. Urša Pečar Fonović, Ana Mitrović, Damijan Knez, Tanja Jakoš, Anja Pišlar, Boris Brus, Bojan Doljak, Jure Stojan, Simon Žakelj, Jurij Trontelj, Stanislav Gobec, Janko Kos, "Identification and characterization of the novel reversible and selective cathepsin X inhibitors", *Sci. rep.*, **7**, 11459, 2017.
12. Milica Perišić, Jerica Sabotič, Urban Švajger, Anahid Jewett, Janko Kos, "Cystatin F affects natural killer cell cytotoxicity", *Front. immunol.*, **8**, 1459, 2017.
13. Anja Pišlar, Biljana Božić, Nace Zidar, Janko Kos, "Inhibition of cathepsin X reduces the strength of microglial-mediated neuroinflammation", *Neuropharmacology*, **114**, 88-100, 2017.
14. Monika Primon, Peter C. Huszthy, Helena Motaln, Krishna M. Talasila, Hrvoje Miletic, Nadia A. Atai, Rolf Bjerkvig, Tamara Lah Turnšek, "Cathepsin L silencing increases As2O3 toxicity in malignantly transformed pilocytic astrocytoma MPA58 cells by activating caspases 3/7", *Exp. cell res.*, **356**, 1, 64-73, 2017.
15. Sonja Prpar Mihevc, Mojca Pavlin, Simona Darovic, Marko Živin, Matej Podbregar, Boris Rogelj, Tomaž Marš, "Modelling FUS mislocalisation in an in vitro model of innervated human muscle", *J. mol. neurosci.*, **62**, 3/4, 318-328, Aug. 2017.
16. Mateja Prunk, Janko Kos, "Nanoparticle based delivery of protease inhibitors to cancer cells", *Curr. med. chem.*, **24**, 42, 4816-4837, 2017, .
17. Nina Recek, Matic Resnik, Rok Zaplotnik, Miran Mozetič, Helena Motaln, Tamara Lah Turnšek, Alenka Vesel, "Cell proliferation on polyethylene terephthalate treated in plasma created in SO_2/O_2 mixtures", *Polymers (Basel)*, **9**, 3, 82, 2017.
18. Katja Škrlec, Anja Pucer Janež, Boris Rogelj, Borut Štrukelj, Aleš Berlec, "Evasin-displaying lactic acid bacteria bind different chemokines and neutralize CXCL8 production in Caco-2 cells", *Microb. biotechnol.*, **10**, 6, 1732-1743, 2017.
19. Maja Zakošek, Janko Mrkun, Breda Jakovac-Strajn, Katarina Pavšič Vrtač, Janko Kos, Anja Pišlar, Petra Zrimšek, "The influence of macro- and microelements in seminal plasma on diluted boar sperm quality", *Acta vet. Scand.*, 11, **59**, 1-9, 2017.
20. Gašper Žun, Janko Kos, Jerica Sabotič, "Higher fungi are a rich source of L-amino acid oxidases", *3 biotech*, **7**, no 3, 230, 2017.
21. Simon Žurga, Milica Perišić, Janko Kos, Jerica Sabotič, "Fungal lectin MpL enables entry of protein drugs into cancer cells and their subcellular targeting", *Oncotarget*, **8**, 26896-26910, 2017.

Review Article

1. Barbara Breznik, Helena Motaln, Tamara Lah Turnšek, "Proteases and cytokines as mediators of interactions between cancer and stromal cells in tumours", *Biol Chem*, **398**, 7, 709-719, 2017.
2. Andrea Markovinovic, Raffaello Cimbro, Tereza Ljutic, Jasna Kriz, Boris Rogelj, Ivana Munitic, "Optineurin in amyotrophic lateral sclerosis: multifunctional adaptor protein at the crossroads of different neuroprotective mechanisms", *Prog. neurobiol.*, **154**, 1-20, 2017.
3. Sonja Prpar Mihevc, Simona Darovic, Anja Kovanda, Ana Bajc Česnik, Vera Župunski, Boris Rogelj, "Nuclear trafficking in amyotrophic lateral sclerosis and frontotemporal lobar degeneration", *Brain*, **140**, issue 1, 13-26, 2017.

Patent

1. Mojca Lunder, Matjaž Ravnikar, Borut Štrukelj, Aleš Berlec, Boris Čeh, *Modified food grade microorganism for treatment of inflammatory bowel disease,* EP2521737 (B1), European Patent Office, 30. 08. 2017.
2. Katja Žmitek, Nataša Tavčar, Tina Pogačnik, Janko Žmitek, Petra Keršmanc, Tadej Rejc, Uroš Petrič, Borut Štrukelj, Samo Kreft, *Extract from the wood of trees of the genus fir to prevent, alleviate or treat unwanted skin changes and preparations,* SI25053 (A), Urad RS za intelektualno lastnino, 31. 03. 2017.

Mentoring

1. Ana Mitrović, *Role of cysteine cathepsins B and X and their inhibitors in epithelial-mesenchymal transition of tumor cells:* doctoral dissertation, Ljubljana, 2017 (mentor Janko Kos).