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The possible modulatory effect of metallic nanoparticles on macrophage polarization

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Macrophages in general can be polarized into either classically activated (M1) or alternatively activated (M2) phenotypes, which differ in cell surface markers, secreted cytokines, and biological functions. Tumor-associated macrophages (TAMs) - as a result of re-programming driven by tumor and other tumor-stromal cells - can create and maintain an immunosuppressive environment and promote tumor malignancy. The disruption of communication between tumor cells and TAMs could be the base of an efficient anti-cancer strategy to prevent tumor progression. Our main goal was to investigate whether the presence of gold (AuNPs) or silver (AgNPs) nanoparticles could interrupt the biological interactions between cells and possibly interfere with the polarization process, thus may modulate the tumor promoting effect of TAMs. Using 4T1 breast cancer and J774 macrophage murine cell lines, this issue was addressed in two *in vitro* systems: 1. We applied the nanoparticle-conditioned medium (CM) of 4T1 cells on J774 macrophages; 2. Tumor cell-macrophage co-culture system was established in which the tumor cells were treated with AgNPs or AuNPs. The expression of certain M1 and M2 polarization markers was assessed by RT-qPCR. Both CM treatments and co-culturing of macrophages and tumor cells show significant impact on the expression levels of M1 and M2 markers detected in macrophage cells. Our results show that nanoparticle treatments could possibly moderate M2 polarization in co-culture system since altered expression of polarization markers was detected upon metal nanoparticle treatments compared to the untreated cells. Moreover, we observed a drastically elevated migratory capacity of J774 macrophage cells upon CM treatments and co-culturing with tumor cells, which was significantly reduced by gold and silver nanoparticle exposures. The gelatin zymography results show a higher secretion of matrix metalloproteinases (MMPs) by J774 cells in both *in vitro* systems, however, AuNPs or AgNPs did not modify this elevated secretion. The increased migration and MMP secretion are usual characteristics of TAMs, and although nanoparticles did not affect the MMP secretion, they had a suppressing effect on macrophage migration. Based on our results, these *in vitro* models are adequate for investigating the molecular interactions between macrophages and tumor cells. Besides the intrinsic anti-cancer effects of metal nanoparticles, AgNPs and AuNPs represent indirect anti-tumoral effect via affecting the migration and the polarization of tumor-associated macrophages, which can be exploited to enhance tumor therapy.

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Unraveling the role of nodule-specific GRPs in nitrogen-fixing symbiosis

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To understand the molecular mechanisms leading to symbiotic nitrogen-fixing root nodules in legumes, many studies were performed to identify nodule-specific genes and gene families. Among them two nodule-specific gene families encode secreted peptides, namely glycine-rich proteins (nodGRPs) and the cysteine-rich peptides (NCRs). These symbiotic peptides have evolved exclusively in the IRLC legumes, and ~700 NCR genes and 34 GRP genes were identified in the *Medicago truncatula* genome that are active at different stages of symbiotic nodule development. While the role of NCRs in the terminal differentiation of bacteroids in nitrogen fixing root nodules has been demonstrated, little is known about the possible symbiotic functions of the nodGRP peptides. GRPs have been described in a wide variety of plant species with a glycine content of 80% ordered in typical repeated motifs, that perform variable roles often in biotic and abiotic interactions between plants and their environment. The nodGRPs are shorter and contain less glycine, without clear motif structures and only produced during root nodule development.

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Our aim in this work is to understand the symbiotic function of two members of the MtnodGRP family by using different experimental approaches. Firstly, by analyzing the symbiotic phenotype of mutant *Medicago* lines that has a gene silencing of MtnodGRPs. RNAi constructs were used to generate *M. truncatula* transgenic lines with reduced expression of targeted MtnodGRP genes. We detected impaired symbiotic nodule structure on the roots of individuals from two transgenic RNAi lines. Other part of the work was about Investigating the regulation of MtnodGRP genes in symbiotic Medicago roots by preparing several constructs to study the promoter activity. The activity of the constructs has been tested in transgenic hairy roots on *M. truncatula* plants. We have also produced overexpressing constructs for some of the MtnodGRPs to test their effect on the nodulation and was done also in transgenic hairy roots on *M. truncatula* plants. Main part of the work was also to identify the interacting partners of the GRPs inside the symbiotic cells by generating constructs of MtnodGRP genes encoding tagged recombinant proteins to help the identification of candidate interacting partners of the MtnodGRPs with pull-down experiments and their localization in the symbiotic nodule cells.

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Engineering bacteria and bacteriophages for biotechnological purposes

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The main field of my work is the thoughtful and planned modification of microbiota to design cell lines or bacteriophages that can produce commercially valuable compounds and could be put to industrial or pharmaceutical use later. We are working on the application of bacteriophages presenting agonistic peptides for targeted activation of G-protein coupled receptors.

G-protein coupled receptors (GPCRs) are cell surface proteins with transmembrane domains, whose role has long been known in the transmission of extracellular signals to signal transduction pathways within the cell and thus in triggering the cellular response. Their ligands are extremely diverse, ranging from photons to small molecular compounds and larger proteins. Recently, their role in the regulation of the cell cycle and thus in tumor formation, angiogenesis and metastasis has emerged. The subject of this study is the development of recombinant bacteriophages that present agonistic peptides on their surface to activate various GPCRs. In this way, not only a cheap and flexibly designed experimental system can be set up for the investigation of GPCRs, but it is also possible to develop a versatile new tumor prevention and treatment strategy.

We decided to examine two endogenous peptides: endomorphin-2 and metenkephalin. These peptides are agonists of pharmacologically relevant μ-opioid receptors belonging to the family of GPCRs. The selected bacteriophage is K1F belonging to the T7 family, which specifically infects (mostly pathogenic) E. coli bacterial strains with a K1 capsule. To "decorate" bacteriophage capsids with opioid peptides we utilized the Spytag-Spycatcher system. We modified the K1F phage genome to express the Spytag protein on its small capsid subunit thus creating a "scaffold" bacteriophage that can present various peptides on its surface. Additionally, we are expressing the opiopeptide-Spycatcher fusion proteins in *E. coli* and upon purification, The phages could be decorated via spontaneous covalent bonding of the Spytag-Spycatcher protein pair.

The bacteriophages presenting endomorphin-2 and metenkephalin respectively were examined in receptor binding assays and showed greater affinity towards their respective receptors than the unbound opiopeptides. A possible explanation for this phenomenon is that the phage can bind up to 20 opiopeptide molecules through the Spytag-Spycatcher system and thus, they will present a locally concentrated "bouquet" to the GPCRs.

Our results show that there is potential in using bacteriophages as delivery vessels for GPCR agonist peptides, paving the way towards a new type of medicine targeting system.

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Identification, structural analysis, and biological activity of non-ribosomal fungal peptides

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Filamentous fungal species from the genus *Trichoderma* play an important role in various fields of agriculture and biotechnology, and one of their important secondary metabolic products are the peptaibols. Peptaibols can be characterized by a high number of non-proteinogenic amino acids in their sequences and the variability of amino acids, due to the modular structure of the producing non-ribosomal peptide synthetases (NRPSs). Peptaibol extracts are capable of inhibiting plant pathogenic Gram-positive bacterial and several fungal species, thus, they can have a role as biocontrol and plant growth supporting agents. In addition to laboratory tests and experiments on plants with peptaibol extracts, modern computer modeling techniques, such as accelerated molecular dynamics (aMD) simulations can help to provide a deeper insight into the mechanism of action of peptaibols.

The aim of our work was to determine peptaibols of 6 *Trichoderma* species from clade Longibrachiatum, of two *Trichoderma rossicum* strains from clade Stromaticum and of 4 *Trichoderma* strains from clade Harzianum. Purified peptaibol extracts were tested against commonly known six Gram-negative and five Gram-positive bacterial strains, as well as four plant pathogenic fungal species. Minimum inhibitory concentration (MIC, mg ml^{-1}) and effective concentration (EC, mg ml^{-1}) values of the purified peptaibol extracts were determined using *in vitro* tests. Based on our results, the peptaibol extract of the clinical isolate *Trichoderma longibrachiatum* f. *bissettii* SZMC 12546 exerted a strong inhibitory effect against most of the Gram-positive bacteria and showed an inhibitory effect against the Gram-negative *Rhizobium radiobacter* (formerly known as *Agrobacterium tumefaciens*). Out of the peptaibols of the two *T. rossicum* strains, the longer peptaibol sequences exerted the stronger inhibitory effects against Gram-positive bacteria as well as the Gram-negative *R. radiobacter*.

To gain deeper insight into the mechanism of the effects exerted by peptaibols, aMD simulations were carried out with the peptaibol sequences produced in the largest quantities. To understand the structure-activity relationships (SARs) of peptaibols, we compared the *in vitro* MIC and EC values with our results obtained during the simulations and looked for correlations between the folding mechanisms, structural properties of the peptaibols and their exerted bioactivities. Based on our results, several defining structural properties, such as the amino acid composition or the location of hydrophobic and hydrophilic regions and the peptaibol length, can influence the expressed bioactivity. In the future, our results can lead to the prediction of the effects of peptaibol extracts based on modeling results.

To compare the previous SARs results with the growth-supporting effect of peptaibols, we selected peptaibol extracts for further experiments on plants under plant growing chamber, greenhouse, and field conditions. The comprehensive experiments on peptaibols may lead to an effective design of peptaibiotic intervention required for plant disease management and can also facilitate the potential future application of peptaibols in agriculture.

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The role of ROP GTPase signaling in plant meristem function

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Our current goal is the identification of substrates for the *Arabidopsis* ROP-activated receptor-like cytoplasmic kinases (RLCK VI_A). These kinases were discovered in our laboratory as the only known ROP-effector kinases in plants.

Potential substrates of RLCK VI_A2 kinases were previously selected based on a yeast 2-hybrid screen and a phosphoproteomic approach. One of the candidate substrates is KNAT5, which is a member of the class II KNOX transcription factors. KNAT5 is known to affect meristem development in antagonism with BELLRINGER 1 (BLR1), another meristem-regulating transcription factor we have demonstrated to control the expression of RLCK VI_A2. Therefore, we hypothesize that BLR1 might antagonize KNAT5 function via RLCK VI_A2. This is supported by the observed weak meristem-related phenotypes

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of RLCK VI_A2 mutants/overexpressors.

In our experiments, we intended to prove that KNAT5 is a potential RLCK VI_A2 substrate using wild-type and kinase-dead RLCK VI_A2 variants. For in planta assays, we cloned the two kinase and substrate proteins into an *Agrobacterium* vector. The constructs have been transfected into an appropriate *Agrobacterium* strain, allowing the transient transfection of *Arabidopsis* seedlings according to the FAST technique. This technique is based on the cocultivation of young plant seedlings with *Agrobacterium* in the presence of a surfactant. The young seedlings are susceptible to *Agrobacterium*-mediated transfection and strongly express the genes cloned into the gene transfer vector within a few days. Since the kinase and substrate genes are on the same vector, their co-expression is ensured. The two proteins are labeled by different tags (9xMyc and 3xHA, respectively). After testing the success of the transformation by Western blot, we have run the extracts on a PhosTag gel to examine the possible phosphorylation of the substrate if co-expressed with the wild type but not the kinase-dead kinase. The protein interaction has also been tested by co-immunoprecipitation. The experiments are running in three independent repetitions. Phenotypic analysis of kinase mutant and overexpressor plants in the wild-type and blr1 mutant background runs in parallel.

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High-content imaging platform for 3D cell cultures using light-sheet microscopy

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Over the last decades, three-dimensional (3D) cell cultures (i.e. spheroids, organoids, microtissues, aggregates, and in general 3D co-cultures) have gained increasing attention in cancer research, regenerative medicine, and drug discovery as they properly reflect the tumor-specific microenvironment. However, the limited imaging depth of confocal microscopes and the lack of high-content screening capabilities are significant obstacles to exploiting inherent possibilities. In this project, we established a pipeline, that comprises all the steps required to evaluate 3D cell cultures at a single-cell level. The novelty of the pipeline is the custom-made 3D-printed imaging plate that is compatible with the already existing Leica SP8 TCS DLS and Stellaris DLS microscopes without any further modifications. The plate provides high penetration depth that reached 250 μm for optically cleared human cancer spheroids. Thanks to the innovation, the proposed concept is cost-effective, semi-automated, easily customizable, and especially designed for high-content screening. Furthermore, the deep learning-based 3D nucleus segmentation provides quantitative evaluation of the data at single-cell resolution that ensures wide-range applicability of the proposed pipeline. Such a system is particularly useful for industrial drug screening, personalized medical services, as well as scientists and pathologists working with 3D cell cultures or 3D tissue sections. This working pipeline may enable important advances in drug discovery and regenerative medicine.

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The role of ZBTB1 at the stalled replication fork

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During S phase, when the genome is especially vulnerable, lesions can block replication because the replicative polymerase cannot incorporate a nucleotide opposite the damaged site. Prolonged stalling of the replication fork can lead to double strand breaks and genomic instability. The RAD18-dependent DNA damage tolerance (DDT) pathways enable the completion of replication through damaged sites via translesion synthesis (TLS) or template switching (TS). At the stalled replication fork, PCNA, the processivity factor of the replicative polymerase, can be mono- or polyubiquitylated. The different posttranslational

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modifications of PCNA determine the choice between TLS and the template switching process. Monoubiquitylation of PCNA by the Rad6 and Rad18 proteins promotes the recruitment of TLS polymerases to the damaged site. These polymerases can insert nucleotides opposite the lesions, although most likely in an error-prone manner. PCNA also can be polyubiquitylated, which promotes the reversal of the replication fork, where the newly synthesized DNA strand will serve as a template for DNA synthesis, leading for and error-free rescue of the replication fork.

ZBTB1 plays an important role as an upstream regulator of the RAD18-dependent DNA damage tolerance pathways. ZBTB1 promotes PCNA monoubiquitylation through chromatin remodelling. We examined the direct interaction between Rad18 and ZBTB1. We also generated mutations in the functional domains of ZBTB1, both the ubiquitin binding UBZ4 domain and the BTB domain is necessary for the function of ZBTB1 in the DDT.

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Interaction of the sorting nexin 25 homolog Snazarus with Rab11 balances endocytic and secretory transport and maintains the ultrafiltration diaphragm in nephrocytes

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Proper balance of exocytosis and endocytosis is important for the maintenance of plasma membrane lipid and protein homeostasis. This is especially critical in human podocytes and the podocyte-like *Drosophila* nephrocytes that both use a delicate diaphragm system with evolutionarily conserved components for ultrafiltration. Here we show that the sorting nexin 25 homolog Snazarus (Snz) binds to Rab11 and localizes to Rab11-positive recycling endosomes in *Drosophila* nephrocytes, unlike in fat cells where it is present in plasma membrane/lipid droplet/ER contact sites. Loss of Snz leads to redistribution of Rab11 vesicles from the cell periphery and increases endocytic activity in nephrocytes. These changes are accompanied by defects in diaphragm protein distribution that resemble those seen in Rab11 gain-of-function cells. Of note, co-overexpression of Snz rescues diaphragm defects in Rab11 overexpressing cells, whereas snz knockdown in Rab11 overexpressing nephrocytes or simultaneous knockdown of snz and tbc1d8b encoding a Rab11 GAP led to massive expansion of the lacunar system that contains mislocalized diaphragm components: Sns and Pyd/ZO- 1. We find that loss of Snz enhances while its overexpression impairs secretion, which, together with genetic epistasis analyses, suggest that Snz counteracts Rab11 to maintain the diaphragm via setting the

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Characterization of developmentally expressed unannotated genes, which regulate fruiting body morphogenesis of the Basidiomycota model organism *Coprinopsis cinerea*

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The morphogenesis of fruiting bodies is a complex process in fungal development, determined by a genetically encoded programme that has reached the highest level of complexity in the Agaricomycetes. According to the literature, thousands of genes are involved in this process in the Basidiomycota model organism *Coprinopsis cinerea*. While previous studies have described numerous novel conserved gene families related to morphogenesis, more recent reports indicate the existence of conserved, developmentally expressed, unannotated gene groups encoding proteins without known conserved domain signatures, possibly forming novel gene families. A subset of these genes is particularly interesting because they are significantly upregulated during primordium initiation or sporogenesis. Therefore, in the present work I have attempted to investigate the function of

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selected unannotated genes, using reverse genetics.

To select the most relevant genes for disruption, I combined previously published gene expression datasets of *C. cinerea* fruiting body development with a recent recently conducted RNA-Seq analysis of sporogenesis. I selected three genes with predicted 7 transmembrane helices (p7TM) that are upregulated in the primordium and later stages of fruiting body development compared to vegetative mycelia, and another three genes that have high expression levels only in the gills during sporogenesis (putative genes whose absence may cause "sporelessness", pSL). For the deletion of the genes, I used the CRISPR/Cas9 system. According to the preliminary results, the observed phenotypes of the knock-out mutants of the p7TM gene showed various developmental changes, ranging from minor defects (dense primordia production) to more severe malformations (absence of lamellae). One of these is particularly interesting: the deletion mutant produces spherical, snowball-like structures with reduced cap and rudiment inner structures. This gene was named SNB1. This gene is conserved in all agaricomycete species studied and they show the same expression pattern during their development. This observation suggests that this gene plays a fundamental role in regulating development in mushroom-forming species.

So far, the disruption of two of the three pSL genes has been successful. All mutants showed the phenotype of a white cap, hence the absence of spore production. Otherwise, these mutants showed no other morphological differences from the wild type, making them suitable candidates to produce sporeless industrial fungal strains.

These results demonstrate that several conserved, developmentally expressed unannotated genes are involved in morphogenesis and their study may reveal exciting new patterns in fungal development.

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Construction of ORFeome based two-hybrid libraries of *Sinorhizobium meliloti*: identification of putative bacterial targets of the symbiotic plant peptide NCR247

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The gene repertoires have been determined for multiple *Sinorhizobium meliloti* strains, which can be symbiotic partners of *Medicago*, *Melilotus* and *Trigonella* species. The functional annotation of the genes has been achieved based on the homology of their predicted products to known proteins or by investigating cellular phenotypes after altering the cellular level of proteins by knocking out or overexpressing the corresponding genes. Proteins act usually in complexes and their interactions with other polypeptides may change or be required for their activity. The identification of these interactions may reveal important clues about protein function and contribute to a more complete functional annotation. We report here the construction of a bacterial and a yeast two-hybrid prey library containing each member of the complete ORFeome of *S. meliloti* strain 1021. The functionality of this new genomics resource has been proved with the detection of known and predicted protein-protein interactions, as well as by screening the putative bacterial protein targets of the symbiotic plant peptide NCR247 affecting bacteroid differentiation via multiple mechanisms.

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Multiplex immunophenotyping in chronic obstructive pulmonary disease and lung cancer

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Chronic obstructive pulmonary disease (COPD) is an incurable inflammatory lung disease, patients have emphysema and chronic bronchitis at the same time. The progression of COPD may cause adenocarcinoma. The main risk factor of COPD is

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tobacco use, however around 25-45% of COPD patients were never-smokers. COPD and adenocarcinoma are the top of the leading causes of deaths worldwide.

We defined 4 experimental patient groups: smoking healthy control (n =10), stable COPD (n =12), exacerbated COPD before steroid treatment (n=11) and adenocarcinoma patients before chemotherapy (n =10). We collected peripheral blood mononuclear cells (PBMC) and plasma samples by gradient separation. We examined the effects of tobacco smoking for the immune system by mass cytometry in all examination groups using Human Immune Monitoring antibody panel (Fluidigm). The multiplex Immuno-Oncology Checkpoint protein analysis was carried out from plasma samples using Luminex technology to identify novel therapeutic targets.

Based on our results, we identified significant difference in 8 main immune cell populations (CD11c dim cells, CD4+ T cells, CD8+ T cells, double-negative T cells (DNT cells), monocytoid and plasmacytoid dendritic cells (mDCs, pDCs), monocytes, undefined cell population) and in further subpopulations - 8 metaclusters within B cells, 5 metaclusters within CD4+ T cells. 6 metaclusters within CD8+ T cells, 2 metaclusters within CD11c dim cells, 2 metaclusters within DNT cells, 8 metaclusters within monocytes, 7 metaclusters within natural killer cells (NK cells) and 3 metaclusters in TCRγδ cells. Furthermore, we also identified 17 markers, that differed significantly among the experimental groups: CD185 and HLA-DR in B cells, CD27 and CD127 in CD4+ T cells, CD8, CD25 and CD27 in CD4+CD8+ T cells, CD16, CD45, CD197 in CD4+ NKT cells, CD27, CD28, CD127, CD183 and CD197 in CD8+ T cells, CD16, CD56 and CD183 in CD8+ NKT cells, CD28 and CD183 in DNT cells, HLA-DR, CD3 and CD14 in mDCs, CD4 and CD11c in monocytes, CD56 in NK cells, CD19, CD27, CD38, CD45 and CD45RA in plasmablasts, CD183 in TCRγδ cells.

Furthermore, we sorted two interesting memory T cell populations, which showed significant difference among groups. The transcriptome of these particular CD4+ effector and central memory T cell subsets have compared by MACE RNA sequencing. The multiplex immuno-oncology checkpoint protein panel showed 17 soluble proteins (BTLA, CD27, CD28, TIM-3, HVEM, CD40, GITR, LAG-3, TLR2, GITRL, PD-1, CTLA4, CD80, CD86, PD-L1, PD-L2, ICOS) significantly increased in patient groups.

Our results revealed changes in immune regulation in chronic inflammation and lung cancer.

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Predicted dimerization of certain LBD transcription factors expressed in *Brachypodium distachyon* based on molecular dynamics

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The subject of my research is the LDB transcription factor family of *Brachypodium distachyon* (purple false brome) the model organism of monocot plants. The LBD proteins belong to the leucine-zipper type transcription factors. The molecular mechanisms of dimerization and DNA binding have been described earlier (Chen et al. 2019) on the Ramosa2 5ly0, one of the LBD proteins of wheat. The overall conservation of the protein sequences indicate that other LBD proteins share these mechanisms and allows us to model other LBDs.

One of the conclusions of the Ramosa2 structure determination was that the exact amino-acid sequence greatly affects the partner-specificity of the leucine-zippers. This funding is supported by *in silico* molecular dynamics as well. Characteristic feature of the LBD transcription factors is a conserved cysteine residue at the neck area (the N-terminal end of the leucine zipper). In the 5ly0 dimer model available in the RCSB protein database, the SH-sidechains of these Cys residues point towards each other in a manner that raises the possibility of a structure stabilizing disulfide bridge between the two monomers.

The current aim of my research is to model the 3D structure and the dimerization mechanism and the possible monomer combinations of the LBD sub-family 1 in *Brachypodium*, using molecular dynamics, a method of simulating the conformational changes of a molecule over a time period, creating a trajectory in a stepwise fashion. I used the *in vitro,* (using X-ray crystallography) experimentally determined structure of the Ramosa 5ly0 as a template for the 3D *in silico* modelling of the leucine zipper domain of *Brachypodium* LBD 1 proteins as well as the starter conformation of each combination of two monomers. I made batches of 10 molecular dynamics simulations using the 5ly0 reference template for the starting conformations.

Three different parameters were used to describe the probability / likeliness of dimer combinations. The first one was the

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average RMSD value of trajectories RMSD functions, the second one is the average distance of the conserved cysteine S-atoms throughout the trajectories. The third parameter is the average difference between the alpha C-atoms' pairwise distances of the various conformations in all models compared to the 5ly0 PDB model. Based on these three parameters, I can classify the dimer-combinations into different probability groups (likely, uncertain, not likely to dimerize). Furthermore, I will use the most probable combinations to make molecular dynamics simulations with models that include the DNA-binding head domain.

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Characterization of the virulence genes of *Mucor lusitanicus*

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Mucor lusitanicus is an opportunistic human pathogenic fungus causing a frequently fatal infection, mucormycosis, in immunosuppressed patients. It is a dimorphic filamentous fungus that shows multi-budded yeast growth form in the presence of a fermentable hexose under anaerobic conditions and filamentous growth under aerobic conditions or upon nutrient limitation. It is resistant to most clinical antifungals. Transcriptomic analyses of *M. lusitanicus* cultures transferred from aerobic to anaerobic conditions revealed 539 differentially expressed genes, including 190 upregulated and 349 downregulated transcripts. *Hsf1, hsf2, qdr2a, qdr2b, qdr2c* and *qdr2d* genes were selected for further investigations. They were upregulated under anaerobic conditions.

Fungal heat shock transcription factors (HSFs) are essential for stress response. Quinidine resistance 2 (QDR2) transporter is involved in the export of quinidine, barban, cisplatin and bleomycin in *Saccharomyces cerevisiae*. It has a role in cation homeostasis, oxidative stress response and biofilm formation in *Candida glabrata.*

Hsf1 was upregulated at 20 °C and *hsf2* was highly expressed at 35 °C. Expression of *qdr2* genes were analyzed after antifungal treatment (ravuconazole, isavuconazole, voriconazole, itraconazole, posaconazole, fluconazole, ketoconazole, and amphotericin B). *Qdr2a, qdr2b and qdr2c* were upregulated in response to all tested antifungals. Transcript level of *qdr2d* increased significantly after treatment with voriconazole, itraconazole, posaconazole, fluconazole, ketoconazole, and amphotericin B. *Hsf1, hsf2, qdr2a, qdr2b, qdr2*c and *qdr2d* single knock-out mutants of *M. lusitanicus* were created by using a plasmid free CRISPR-Cas9 system. The double-strand break caused by the Cas9 enzyme was repaired by homologous-directed repair. *Hsf1* and *hsf2* deletion mutants showed significantly decreased colony growth comparing to the control strain at 15 and 20 °C. *Hsf2* deletion mutant also displayed tendency to grow significantly weaker than control strain at 30 and 35 °C. Disruption of *hsf* genes caused weaker growth of *Mucor* in the presence of SDS. Disruption of *qdr2* genes has no effect on the antifungal susceptibility and growth in the presence of cell wall stressors. Spore production ability of the *hsf1* and *hsf2* deletion mutants did not alter from that of the control strain, while *qdr2* deletion mutants showed significantly lower sporulation. In the *in vivo Galleria mellonella* model, disruption of *hsf1* and *hsf2* did not affect virulence of *M. lusitanicus,* while deletion of *qdr2a*, *qdr2b*, *qdr2*c and *qdr2d* led to significantly decreased virulence.

These results suggest that *hsf1* and *hsf2* are involved in the response to different temperatures. *Qdr2* genes have a role in the pathogenicity of *M. lusitanicus*. The loss of *qdr2a, qdr2b, qdr2c* and *qdr2d* genes may be compensated by each other since they have overlapping expression patterns. The study was supported by the grants NKFI K131796, ELKH2001007 and ITM NKFIA TKP-2021-EGA-28.

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The *Arabidopsis* mitochondrial protein PPR40 modulates drought tolerance

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Plants exhibit various alterations in their growth and development in response to adverse environmental conditions. Water deficit and salt stress may interfere with photosynthesis and enhance photorespiration, thereby altering normal cell homeostasis and increasing ROS production, especially under high light intensity or in combination with other stresses. ROS can damage proteins, lipids, and other macromolecules and therefore create additional oxidative stress for the plants. In response to these stresses, plants have developed various mechanisms to adapt and survive. These include changes in gene expression, production of protective compounds such as antioxidants, and alterations in growth and development. Genetic screening for mutants with increased sensitivity to abscisic acid (ABA) led to the identification of ppr40- 1 mutation. However, subsequent research revealed that the mutation also led to delayed germination, semi-dwarf growth habits, and increased salt sensitivity. This pleiotropic phenotype indicates that the disruption of mitochondrial electron transport caused by the inactivation of PPR40 may have an impact on various cellular functions and stress responses (Zsigmond et al. 2008).

In this study, we investigated how drought can affect plant metabolism and physiology in ppr40 mutants of *Arabidopsis thaliana*. Studies in *Arabidopsis thaliana ppr40-1* (knockout), *ppr40-2* (leaky mutation) mutants and Col-0 has shown that *ppr40-1* has higher survival rates when subjected to drought. Enhanced drought tolerance of *ppr40-1* mutant was confirmed by various parameters like higher electron transport rate, lower proline and malondialdehyde contents (lipid peroxidation level) and higher relative water content compared to wild type (Col-0). Drought related genes like *P5CS1*, *RD29A*, *RAB18* has also shown lower expression in our semi-dwarf mutant *ppr40-1* compared to *ppr40-2* mutant and Col-0. With the use of state of art complex phenotyping system, we measured green area, compactness, area ratio, slenderness of leaves, different chlorophyll fluorescence parameters (maximum and actual quantum yields, NPQ, YNO etc.) and validated our studies. Plants are capable of synthesizing ABA in drought stress and hence able to close their stomata to decrease the rate of transpiration. Our mutant can close its stomata as it is very sensitive to ABA. The possible explanation of our studies that *ppr40-1* is retaining higher amount of water is faster closure of stomata. Hence, the *ppr40-1* mutant is resistant to drought. Advancements in the comprehension of PPR proteins and their pivotal role in plant responses to abiotic stresses have the potential to pave the way for the development of innovative strategies aimed at enhancing plant tolerance to environmental adversities, thus contributing to the establishment of sustainable agriculture practices.

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Antimicrobial activity of γ-core motif peptide derivatives of antifungal proteins from filamentous Ascomycetes

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Because of the emerging number of drug-resistant fungal strains, the incidences of fungal infections dramatically increased worldwide. Therefore, there is an urgent demand for new compounds with different antifungal mode of action from that of the conventional antifungal drugs. Antimicrobial peptides are potential candidates. Up to date, huge numbers of antimicrobial peptides have been investigated from both natural and synthetic sources and have shown the ability to kill fungal pathogens. A detailed phylogenetic analysis indicated the presence of different antifungal proteins (AFPs) in Eurotiomyces. In spite of the high differences, all of them contain the evolutionary conserved so-called γ-core motif with $GXCX_{[3-9]}$-C amino acid order, where X can be any amino acid. The synthetic peptide derivatives spanning this motif can effectively inhibit the fungal growth, therefore, they also represent antifungal drug candidates. In the present study, we investigated how the physicochemical properties of the γ-core motifs influence their antifungal efficacy. We investigated the antifungal efficacy of 21 γ-core peptide derivatives (P_{γ}s) of AFPs with different physicochemical properties against four filamentous fungi (*Aspergillus fumigatus* CBS

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101355, *Botrytis cinerea* SZMC 21472, *Cladosporium herbarum* FSU 1148, *Fusarium oxysporum* f. sp. *lycopersici* CBS 123668), and two yeasts (*Candida albicans* SC5314, *Saccharomyces cerevisiae* SZMC 0644). Antifungal susceptibility tests indicated that the antifungal activity of a P_γ highly depends on the fungal species investigated on. We observed that, not just positively charged P_γs are antifungal active as we expected before, but the neutral ones can also have antifungal effect. According to our results, we suppose that the antifungal activity of a P_γ depends on the balance between the charge and the grand average hydropathy value. In the future we would like to test the potential applicability of these peptides in plant and crop protection and investigate their yeast biofilm eradication activity. We would like to reveal the connection between the potential secondary structure and the antifungal efficacy.

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Investigated of continuous hydrogen evolution of *Chlorella* – *Bacillus amyloliquefaciens* inter-kingdom co-cultures in starch-containing media

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We investigated the biohydrogen production of pure and mixed *Chlorella* sp. MACC-360 algae culture with *Bacillus amyloliquefaciens* bacterial partner in three selected media using starch. Synthetic wastewater (SWW), TRIS-Acetate-Phosphate (TAP) and acetate-free TRIS-Phosphate (TP) media was used for the experiments.

The products of starch degradation can serve as extra organic nutrients for photoheterotrophic algae growth. The *B. amyloliquefaciens* bacterial partner was able to completely degrade the applied amount of starch within 48 hours. Axenic algae strains accumulated high amount of starch by the 2nd day, then totally consumed this starch by day 3. The selected bacterial partner plays important role in growth and in algal total hydrogen productivity. Heterotrophic bacteria efficiently consume oxygen during growth, thereby creating ideal conditions for the green algae-based hydrogen production in sealed bottles. The applied pure bacterial strains did not produce hydrogen in three selected media.

The added bacterial partner influenced the pure culture hydrogen production in three selected media. Among the three selected media, the TAP medium gave the best results. In TAP medium, *Chlorella* sp. MACC-360 produced almost 3 times higher amount of hydrogen when co-cultured with bacterial partner compared to the axenic algae cultures both with and without starch. Strong hydrogen peak was showed at day 3 when 24 g L^{-1} starch was added to the TAP medium. Neither the bacterial monocultures nor the axenic algae strains produced any hydrogen in SWW medium, in TP medium with and without starch. However, algal hydrogen production was influenced by *B. amyloliquefaciens* in the starch-containing TP medium. Strong hydrogen peak was showed at day 3 when 8 g L^{-1} starch was added to the TP medium.

The algae monocultures and algal-bacterial co-cultures were tested for continuous biohydrogen production in TAP and in TP media using starch. The fresh media, starch and the bacterium *B. amyloliquefaciens* were added at 72-hour intervals. In TAP medium, all pure and mixed culture was able to produce hydrogen until the end of the 21-day experiment both with and without starch. The added bacterial partner strongly enhanced the pure culture hydrogen production, while the amount of added starch did not further stimulate hydrogen production. In TP medium containing 8 g L^{-1} starch *Chlorella* sp. MACC-360 was able to establish almost stable continuous hydrogen production. Interestingly, when the amount of *B. amlyloliquefaciens* bacterial partner was increased in the co-cultures (to a starting bacterial OD_{600}: 0.175 instead of the earlier applied OD_{600}: 0.07), the hydrogen production of *Chlorella* sp. MACC-360 became stable and continuous in TP medium containing 8 g L^{-1} starch. The results of the acetate-free experiments showed that the ratio of algal and bacterial partners is very important for improving algal hydrogen yield.

The experiments served as a simplified laboratory model for the investigation of the photoheterotrophic algae-based wastewater treatment. We have applied a combination of mixotrophic biodegradation of organic substances and concomitant biohydrogen production in our experimental setup.

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Dental non-metric characteristics of populations from the Carpathian Basin (6–11th centuries CE) in the light of the archaeogenetic data

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Anthropological examination is one of the few research fields that provide invaluable information concerning the biological relationships, and health conditions of past populations. These investigations are mostly based on the analysis of the human skeletal remains, i.e., the bones and teeth.

The tooth contains the hardest tissue of the human body; therefore, they are usually well preserved, even in case of poor taphonomic conditions. Furthermore, after a certain phase of enamel formation, the morphology of the crown is not changing during life, apart from the influence of some external factors (e.g., wear, trauma, and caries). Besides, morphological variations named non-metric traits can appear on the teeth like shovel-shaped incisors formation or Carabelli's cusp which can be studied in historic and prehistoric populations.

In the nineteenth century, dental anthropologists and anatomists described that morphological characters of human dentition vary within and between populations and have patterned geographic variations. Based on the past century's research, it seems dental development is significantly regulated by the action of genes. Therefore, numerous research aimed to investigate the geographical distribution and genetic connections of the non-metric characteristics of human teeth all over the world. To increase the repeatability of the investigation and minimalize inter-observer errors, Turner and his colleagues created a standardized classification system named The Arizona State University Dental Anthropology System (ASUDAS). They have described 36 non-metric dental traits assigned with a scale depending on the degree of expressiveness.

In the last decades, the amount of available archaeogenetic data has increased significantly, providing the opportunity for a comparative analysis with the results of dental non-metric research. However, the number of studies that use the dental non-metric and genetic data of the same individual for comparison is negligible. Additionally, we have little information concerning the Carpathian Basin, as only a few studies have been made about the non-metric characteristics of the teeth from this region. Nonetheless, the number of published archaeogenetic data increased in the last few years, especially from the Avar age (6–9th centuries CE), the Hungarian conquest period, and the early Árpádian-age (10–11th centuries CE) of the Carpathian Basin.

Based on the above-mentioned background, the aim of this study is to collect dental morphological information with the ASUDAS from the 6-11th-century-CE populations of the Carpathian Basin. The research material consists of individuals with published whole genome data. We intend to examine the potential of dental non-metric traits regarding the description of biological relationships between populations from the Carpathian Basin.

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Evolutionary conservation of the metabolome and its links to human diseases

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Metabolite levels play a central role in determining the physiological state of the cell and, as such, alterations in metabolite concentrations can have a considerable effect on the organism, both advantageous and deleterious, *i.e.*, ones that underlie human diseases. However, despite its relevance for human health, to date the evolutionary forces shaping the metabolome remain essentially unexplored. We hypothesize that metabolites differ in their levels of evolutionary conservation due to varying functional constraints that limit the amount of permissible concentration changes that can accumulate during evolution. As a consequence, alterations in the levels of metabolites that are strongly conserved are more harmful. If so, characterizing the evolution of the metabolome would allow us to identify metabolites and metabolic states that underlie human diseases.

To assess the evolutionary patterns of the metabolome, we analyzed a published mammalian metabolomics dataset including concentrations of around 140 metabolites and 120 lipids, from 26 mammalian species. We introduced a score measuring

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the extent of evolutionary conservation for each metabolite and found that the level of conservation varies greatly across metabolites with some showing substantially stronger conservation than others. What determines these differences? We systematically explored the possible determinants of metabolite conservation and discovered two simple properties that explain most of the variation: absolute metabolite concentration and involvement in essential biochemical reactions. Additionally, we used the same multispecies metabolomics dataset to identify evolutionary couplings between pairs of metabolites. Our results show that around 5% of metabolite pairs are evolutionarily coupled, and the concentrations of these metabolite pairs mostly tend to change in the same direction over the course of evolution. This suggests that, at least in some cases, it is the ratio of metabolites, not the metabolite levels themselves that are under strong evolutionary conservation.

Finally, we investigated the evolutionary properties of metabolites that are involved in human diseases. We curated metabolites that are associated with human diseases and found that metabolites associated with four general disease groups were significantly more conserved than others. In the case of metabolic diseases, we showed that evolutionary information alone, without any additional clinical input, predicts this association reasonably well.

Overall, our work uncovers simple rules that govern metabolic evolution in animals and implies that most metabolome differences between species are permitted, rather than favored by natural selection. More broadly, our work paves the way towards using evolutionary information to discover biomarkers, as well as to detect pathogenic metabolome alterations in individual patients.

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Maternal lineages from 10-11th century commoner cemeteries of the Carpathian Basin

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Archaeogenetics is a scientific field that draws conclusions based on genetic material extracted from archaeological findings and most commonly used to uncover the genetic composition and history of ancient populations and cultures.

Hungarian history has been shaped by the Conquerors, who arrived in the Carpathian Basin in the 9th century AD. They established a steppe state and later their descendants the Hungarian Kingdom. The archaeological evidence from elite and commoner cemeteries provides insights into the origins of these populations. Previous archeogenetic studies focused on the elite graves, revealing an admixture of Asian and European groups. They have found that one-third of the Hungarian conqueror elite's uniparental lineages are of Central Asian origin, while two-thirds can be traced back to the Pontic-Caspian steppe region. However, little was known about the genetic composition of the commoners and whether they shared the same origins as the elite.

My doctoral work aimed to investigate the genetic relationship between the commoners and the conqueror elite in the Carpathian Basin. Eight commoner cemeteries dating from the 10th to 11th centuries were selected, and whole mitochondrial DNA (mtDNA) sequences were obtained from 202 individuals via hybridization techniques and new generation sequencing. These sequences were compared to the mtDNA data from the elite graves and other ancient Eurasian populations.

The results showed that the commoners had a distinct genetic composition compared to the elite. The commoners had a higher proportion of west Eurasian haplogroups, while the elite had a more balanced distribution of east and west Eurasian haplogroups. The commoners also showed genetic affinities with ancient European and Near Eastern populations, clustering close to Baltic Bronze Age and Iron Age populations.

The presence of east Eurasian haplogroups in the commoners indicated some level of admixture with the Conqueror elite, possibly through intermarriage or assimilation. The shared haplogroups between the commoners and the elite suggested a reciprocal gene flow between the two groups. However, the commoners also had haplogroups that were not shared with the elite, potentially indicating the influence of previous populations in the Carpathian Basin, such as the Avars.

Population genetic analyses further supported the genetic distinction between the commoners and the elite. Principal component analysis and multidimensional scaling showed that the commoners clustered closer to European populations, while the elite had affinities with both European and Asian populations. The genetic distances between the commoners and the elite

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were significant, indicating that they were genetically distinct groups.

Overall, this study provided insights into the genetic composition of the commoners in the Carpathian Basin during the Hungarian conquest period. The results showed that the commoners had a predominantly European genetic background, with some admixture from the Conqueror elite and maybe some other eastern groups with steppe origins. The findings contribute to our understanding of the complex population dynamics and interactions during this historical period. Further research and analysis of additional genetic markers are needed to gain a more comprehensive understanding of the genetic history of the Hungarian people.

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Regulatory role of lipids during autophagy

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The autophagy pathway is a membrane-driven process where the cellular disposable material is engulfed by autophagosomes to fuse with the lysosome for their degradation. Qa-SNARE Syntaxin17 is one of the indispensable players during the vesicle fusion step which is thought to be recruited to closed autophagosomes from the cytosol. Our previous work could decipher the lipid composition of autophagic membranes. Interestingly, the membranes are abundant with negative lipids such as (phosphatidylinositol) PI, (phosphatidyl serine) PS, (phosphatidic acid) PA, etc. Therefore, we think these negative lipids may have a role to play regarding the autophagosomal recruitment of Syntaxin 17 from the cytosol in its need. Therefore, we investigated the lipid-protein interactions mainly by Liposome flotation assay and Dynamic light scattering (DLS) methods. To characterize the charge of the liposomes we measured their Zeta-potentials. These methods are attained using purified recombinant *Drosophila* Syntaxin 17 (Stx17) and human Syntaxin 17 (STX17) proteins and generated liposomes by natural lipids (total lipids from *Drosophila* and MCF7 [human breast cancer cell line], pig brain or bovine liver) and synthetic lipids (abundant phospholipids in eukaryotic membranes). Liposome flotation assay is an appropriate biochemical method to study the possible specificities of proteins toward certain lipids. The STX17 and Stx17 exhibited general interaction with a wide range of phospholipids but PIs and their phospho derivatives namely PI3P, PI4P, and PI3,5P2 are the most favorable lipids for the protein-lipid interaction. We further moved to the mammalian cell culture, HEK293 cells (human embryonic kidney cells) as our model system to investigate the significant role of PI3P, PI4P, and PI3,5P2 in STX17 recruitment to the autophagosomes. We utilized the advantage of using lipid reporters by transient transfection to show the localization of specific lipids such as pEGFP-2xFYVE (PI3P), GFP-P4M SidM (PI4P), and mEGFP-PI3,5P2 (PI3,5P2) along with RFP-LC3 (autophagy marker protein) and mTorq-STX17. Later treated the cells with chemical inhibitors such as LY294002, NC03, and Apilimod to inhibit the synthesis of PI3P, PI4P, and PI3,5P2 respectively, followed by the fate of STX17 regarding its recruitment to autophagosomes. Our results showed the localization of all 3 PIPs on LC3 and STX17 positive structures but PI4P is most abundant on the matured autophagosomes. However, in the absence of PI4P, STX17 recruitment is prevented. We speculate that electrostatic interactions are developed between negative lipids of the bilayer and basic amino acids of C-terminal STX17. Therefore, in the near future, we generate the amino acid mutations in desired regions of STX17 and investigate the fate of STX17.

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Characterization of the multinucleated giant hemocyte, a novel member of innate immunity

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As innate immune mechanisms are highly similar in insects and vertebrates, *Drosophila* species are frequently used as model organisms to study immune responses. Our research group have previously identified a novel blood cell type, the multinucleated giant hemocyte (MGH) in several *Drosophilids* (Márkus et al. 2015; Cinege et al. 2020), bearing considerable similarities to mammalian multinucleated giant cells, such as the foreign-body giant cell, the Touton giant cell and the osteoclast.

To study the MGHs we have characterized the hemocytes of *D. ananassae* and *Z. indianus* using blood cell type specific monoclonal antibodies, transmission electron microscopy, and transcriptome analysis (Cinege et al. 2020, 2022). MGHs participate in the encapsulation of large foreign particles, like the eggs of parasitoid wasps. Compared to *Drosophila melanogaster,* which develops lamellocytes as encapsulating hemocytes, the species that develop MGHs are more resistant to parasitoids.

The ultrastructural analysis has shown that the cytoplasm of MGHs contains an abundance of different types of vesicles. Upon further investigation using Lysotracker dye we have demonstrated that several vesicles are acidic, and they form an acidic layer on the surface of the parasitoid, which provides an optimal environment for degradative enzymes; a mechanism reminiscent of the mode of action of mammalian giant cells (Ahmadzadeh et al. 2022). We have also described that MGHs release exosomes into the hemolymph, which might play a major role as carriers of toxic effector molecules to the parasitoids.

We have performed a transcriptome analysis of *D. ananassae* blood cells and clustered the genes expressed at high level in MGHs in different functional categories, including vesicle-related, lysosome-related, motility, exocytosis, autophagy. Through this assay we have discovered that these hemocytes express several toxin genes which are known to be characteristic of bacterial organisms, hence possibly originating from prokaryotes by horizontal gene transfer. For example, *cytolethal distending toxin B* (*cdtB*), *apoptosis inducing protein of 56* kDa (*aip56*) (Verster et al. 2023) and *hemolysin E* (*hlyE*) are all encoded in the genome. The latter can be found in 38 different copies, 14 of which are expressed in MGHs. Using qRT-PCR analysis we detected that the expression of these genes was induced by parasitoid wasp infection, suggesting a role in immune defense.

Our research has revealed new insights into the highly effective cell-mediated immunity driven by MGHs, therefore may help to gain a deeper understanding of the mechanisms of analogous mammalian cells, too.

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Analysis of the single-cell heterogeneity of adenocarcinoma cell lines and the investigation of intratumor heterogeneity reveals the expression of transmembrane protein 45A (TMEM45A) in lung adenocarcinoma cancer patients

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Intratumoral heterogeneity (ITH) is responsible for the majority of difficulties encountered in the treatment of lung-cancer patients. Therefore, the heterogeneity of NSCLC cell lines and primary lung adenocarcinoma was investigated by single-cell mass cytometry (CyTOF). First, we studied the single-cell heterogeneity of frequent NSCLC adenocarcinoma models, such as A549, H1975, and H1650. The intra- and inter-cell-line single-cell heterogeneity is represented in the expression patterns of 13 markers—namely GLUT1, MCT4, CA9, TMEM45A, CD66, CD274 (PD-L1), CD24, CD326 (EpCAM), pan-keratin, TRA-1-60, galectin-3, galectin-1, and EGFR. The qRT-PCR and CyTOF analyses revealed that a hypoxic microenvironment and altered metabolism may influence cell-line heterogeneity. Additionally, human primary lung adenocarcinoma and non-involved healthy lung tissue biopsies were homogenized to prepare a single-cell suspension for CyTOF analysis. The CyTOF showed the ITH of human primary lung adenocarcinoma for 14 markers; particularly, the higher expressions of GLUT1, MCT4,

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CA9, TMEM45A, and CD66 were associated with the lung-tumor tissue. Our single-cell results are the first to demonstrate TMEM45A expression in human lung adenocarcinoma, which was verified by immunohistochemistry.

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Focusing on nitric oxide (NO) signalling in strigolactone (SL) mutant and the effect of Zinc (Zn) deficiency in *Arabidopsis thaliana*

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Both NO and SL are growth regulating plant signals. My main experiment was the detect interaction between NO and SLs in *Arabidopsis* wild-type (WT, *Col-0*) and its mutant lines; S-nitrosoglutathione reductase (GSNOR) deficient *gsnor1-3*, GSNOR overproducer *35S:FLAG-GSNOR1*, SL deficient *max1-1*, *max2-1* and *max2-2*. We combined molecular biological and pharmacological approaches (GR24, TIS108, GSNO, cPTIO). We compared the root lengths, the numbers of lateral root primordia and emerged lateral roots. NO levels were detected. S-nitrosothiol (SNO) levels, GSNOR activity and protein abundance were measured. The expressions of NO-associated (NIA1, NIA2, GSNOR1, GLB1, GLB2) as well as SL-associated (CCD7, CCD8, MAX1, MAX2, D14) genes were analysed. We observed that SL-deficiency resulted in elevated NO and SNO levels due to decreased GSNOR protein abundance and activity indicated that there was a signal interaction between SLs and GSNOR-regulated levels of NO/SNO. SL biosynthetic genes (CCD7, CCD8 and MAX1) were down-regulated in *gsnor1-3* contained elevated NO/SNO levels. Based on our results, the sensitivity of *gsnor1-3* to GR24, were suspected that functional GSNOR is needed to be under control. Furthermore, SLs may be involved in GSNO regulated primary root shortening as suggested by the relative insensitivity of *max1-1* and *max2-1* mutants to exogenous GSNO. Our results indicated for the first time connection between SL and GSNOR-regulated NO/SNO signals in *Arabidopsis thaliana* roots.

The involvement of NO signal molecule in Zn-deficiency responses of plants is largely unknown. My second topic was to examine the connection between suboptimal Zn supply and NO signalling. *Col-0* and *35S::FLAG-GSNOR1 Arabidopsis thaliana* were grown under control (15 µM $ZnSO_4$) , mild (1,5 µM $ZnSO_4$) and severe (0 µM $ZnSO_4$) Zn deficient conditions. Zn deficiency caused more changes in root structure of *Col-0.* The morphological changes of root caused by a disturbance in cell division and hormonal changes. The GSNOR enzyme (in both wild-type and *35S::FLAG-GSNOR1* plants) negatively affected by Zn deficiency. The level of NO significantly increased in *Col-0* plants under mild Zn condition.

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K63-dependent ubiquitylation of RNAPII upon DNA double-strand breaks

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Essential biological functions are constantly challenged by genotoxic stresses. During transcription, DNA damage can occur at any stage predisposing to genomic instability through the direct damage to DNA and formation of aberrant/truncated transcripts. A central surveillance mechanism is operated by RNA polymerase II (RNAPII) when it encounters a DNA lesion. If the lesion persists or is difficult to repair as in the case of Double-Strand Breaks (DSBs) then the RNAPII must be removed from the damaged site to allow for DNA repair. The removal and processing of RNAPII is surprisingly a ubiquitylation-dependent mechanism mediated by ubiquitin ligases.

Deregulated ubiquitin ligases (E3s) contribute to uncontrolled proliferation and genomic instability that drive malignant transformation, tumour progression and therapy resistance making them attractive drug targets. Although in recent years

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different E3 ubiquitin ligases have emerged as the preferred choices of big pharmaceutical and biotechnological startups in the context of cancer and disease biology, it appears that the E3 ubiquitin ligases described in this project (WWP2, NEDD4 and CLR3 ubiquitin complex), are not very well recognized yet in the realm of molecular biology and drug discovery. Recent advances have uncovered their involvement during DNA repair and transcription regulation but their exact role, if any, in the clearance of DNA Double-Strand Breaks (DSBs) remains elusive. In this project, we are trying to elucidate the mechanisms of ubiquitylation and identify the biological complexes involved in DSB-induced transcription stress response. The elegance of our designed investigation using novel established practices in our laboratory (AQUA LC-MS/MS, TUBE pull downs, Ubi-CREST, ChIPs) let us uncover that the ubiquitylation of RNAPII upon DSB encounter is proteasomal dependent mediated mainly by WWP2, NEDD4 and CUL3 complexes in a time-dependent fashion. Strikingly, we further observe the presence of K63 ubiquitin chains written on the RNAPII substrate which are mainly involved in DNA repair. Thus, we believe that the ubiquitylation of RNAPII upon DSBs is an integral mechanism of DSB repair pathways, yet this is left to be uncovered. All in all, our project promises to uncover the consequential links between DSB-induced transcriptional silencing, DSB repair, and ubiquitylation and according to this, we hope to unravel key mechanisms that contribute to the pathogenesis of cancer and provide strong basis of targeted cancer therapies.

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A novel role of Protein Phosphatase 4 in DNA damage repair

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Reversible protein phosphorylation regulates complex cellular processes, including cell division, development, or DNA damage response, by modulating the structure, localization, half-life of key proteins. Protein Phosphatase 4 (PP4) is an evolutionarily conserved Ser/Thr and essential phosphatase whose function is poorly understood in vital cellular processes. The major form of PP4, present from yeast to human, is the PP4c-R2-R3 heterotrimeric holoenzyme, in which the R3 regulatory subunit is responsible for target recognition. We and others have shown that the EVH1 domain of the R3 subunit is involved in substrate targeting and binding. Using different biochemical and cell biological approaches, we identified several late-stage DNA repair proteins as interactors (hence putative substrates) of the *Drosophila* PP4R3-EVH1. This includes Centrobin (Ctb) and RAD51 (SpnA), which are known interactors of the BRCA2 tumour suppressor, the key regulator of DNA repair. While the role of RAD51 in this process is well-studied, the function and phospho-regulation of Ctb in DNA repair is not fully understood. We found that depletion of Ctb, PP4c or R3 in human cells, led to a huge lag in DNA repair induced by gamma-irradiation. Moreover, it also caused decrease in homologous recombination frequency and the formation of abnormal chromosomes, which clearly indicate the synergistic role of Ctb and PP4 in this process. Our major aim is to further investigate this novel finding and understand the mechanism of PP4-Ctb-mediated DNA repair.

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Isolation and genetic modification of bacterial viruses to effectively treat nosocomial, antibiotic resistant pathogenic species

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The widespread dissemination of antibiotic-resistant pathogens poses a significant and urgent threat to healthcare. The declining efficacy of both existing and newly developed antibiotics has necessitated the search for alternative strategies. Moreover,

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major pharmaceutical companies have withdrawn from antibiotic discovery efforts due to perceived risks and lack of profitability. One promising alternative to antibiotics is the utilization of bacteriophages (phages), also known as phage therapy, for medical applications. However, a major challenge in the therapeutic use of phages is their narrow host specificity, which is advantageous in targeting specific bacteria without harming beneficial ones but requires the time-consuming generation of phage preparations for almost each treatment. Another emerging limitation is the development of bacterial resistance against phages, which can be overcome by employing phage cocktails (preparations consisting of combination of multiple phages) with broad-spectrum activity and high diversity. Additionally, there is limited research available on the phages present in the human microbiome and their interactions with human cells, which relationship can potentially undermine therapeutic efforts.

Previously, we successfully developed mutagenized hybrid transducing phage particles to deliver metagenomic libraries into clinically relevant pathogenic species such as *Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli*, and other pathogenic strains. Building upon these promising results, our objective was to enhance the therapeutic applicability of naturally occurring phages. To achieve this, we investigated the beneficial components of phages extracted from the human microbiome, focusing on their ability to adhere with the epithelial mucosal surface of the human gut and thus achieve long-term persistence therein. Through bioinformatic analysis of sequenced data from mucus-binding and non-binding phage genomes, we identified several BACON (Bacteroides-Associated Carbohydrate-Binding Often N-terminal) domains belonging to the immunoglobulin-like domain family. We hypothesized that these domains are responsible for the mucin-binding properties of phages associated with humans. As such, we selected four of these domains and employed an established phage engineering system to display them on the minor capsid proteins of K1F phages. Subsequently, we treated mucus producing or non-producing gut epithelial cell lines with the labeled modified phages. Following cellular labeling, we employed fluorescence confocal microscopy to observe the interactions between the BACON-displaying phages and the treated cell lines. Our findings revealed that the presence of BACON domains increased the adhesion and invasion of modified phages to gut epithelial cells compared to wild-type phages.

Our future objectives encompass expanding the repertoire of investigated immunoglobulin-like domains and utilizing them to enhance the therapeutic efficacy of our phages. Additionally, we aim to treat colibactin-producing E. coli strains associated with colorectal cancer using newly isolated phages from sewage water. We also plan to investigate the application of BACON-domain equipped phages against such pathogenic strains on human gut epithelial cells to mitigate the mutational effects of colibactin.

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Spatial profile of calcium transients evoked by backpropagating action potential in human cortical pyramidal dendrites.

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Backpropagating action potentials play important role in synaptic plasticity, dendritic excitability, and compartment specific intracellular Ca^{2+} dynamics. Signal propagation in human dendrites shows potentially species and dendritic region-specific properties and we asked whether action potential backpropagation in human dendrites follows a uniform, or a segment regulated pattern.

We studied human cortical layer 2/3 pyramidal apical dendrites in acute brain slices with somatic whole-cell stimulation and simultaneous dendritic two-photon Ca^{2+} imaging. Single action potentials produced detectable Ca^{2+} influx in segments of apical dendrites up to 270 μm from the soma of human pyramidal neurons. Evoked Ca^{2+} signals showed a stereotyped spatial profile along dendrites: the amplitude of the Ca^{2+} transients increased with distance from the soma, reached the maximum level on the dendritic region 50-100 μm from the soma then decreased towards the distal dendritic regions of primary and higher order dendrites. Non-specific blockage of Ca^{2+} channels and blockade of voltage-gated Na^{+} channels significantly reduced and completely abolished Ca^{2+} transients, respectively. Various Ca^{2+} channel types contributed to the Ca^{2+} signals as shown

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by selective blockade with N-type, L-type, T-type, or R-type Ca^{2+} channel subtypes. These results suggest a booster region in primary dendrites of human pyramidal cells for backpropagation induced Ca^{2+} influx in the dendritic tree.

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Age-related differences in electrophysiological properties of pyramidal cells in the human neocortex

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Several neurological diseases, cognitive impairments, memory loss, and decline in signal processing performance are associated with aging in the human brain. Hence one of the issues in brain research is to understand the physiological processes of neuronal circuits that change with age. Preliminary studies from rodents have revealed differences in electrophysiological properties of cortical pyramidal cells across age groups. Despite the large amount of human data already available, little is known about changes in electrophysiological properties, particularly in the early stages of life. To reveal the potential age-related differences, we investigated the intrinsic membrane properties of pyramidal cells in the human neocortex. We carried out whole-cell patch-clamp recordings in cortical acute brain slices obtained from parietal, temporal and frontal lobe. Our dataset contains more than three-hundred-layer 2/3 pyramidal cells from a wide range of age (0-80 years) divided into predefined groups: infant (<1y), early childhood (1-6y), late childhood (6-12y), adolescence (12-20y), young adulthood (20-40y), middle adulthood (40-60y) and late adulthood (>60y). Pyramidal cells were recorded in whole cell patch clamp mode and stepwise and oscillatory currents were injected to investigate the evoked sub- and suprathreshold membrane potential changes. After the application of an extensive data analysis, we extracted 60 number of features and among them we have successfully identified some properties that differ between age groups. For example, input resistance and time constant are significantly higher in infant patients and decrease with aging, suggesting a remarkable change in neuronal excitability during the process of maturation. In addition, active properties such as action potential half-width, activation speed, and adaptation of the action potential train showed age-dependent differences. These properties may have an impact on signal transmission and synaptic plasticity so these dissimilarities dictate the operation of the cell and can be particularly important at the circuit level. Age dependent differences of electrophysiological features are the result of the morphology and gene expression profile of the cell; therefore, further morphological, and transcriptional investigations are needed for a better understanding of human neuronal aging.

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Interaction of biocontrol bacterial strains and pathogenic moulds

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The white button mushroom (*Agaricus bisporus*) is one of the most frequently cultivated species among mushrooms alongside with oyster mushroom (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*), although growers often have to face damage caused by various pathogens. Pathogenic moulds include *Trichoderma* species causing the so-called green mould disease responsible for the greatest losses, *Lecanicillium fungicola* resulting in dry bubble disease, *Hypomyces perniciosus*, the causal agent of wet bubble disease and *Cladobotryum mycophilum* causing cobweb disease, while among bacteria, *Pseudomonas tolaasii* is responsible for the brown blotch disease.

Continuing the work from the last year we measured the amount of surfactin, fengycin and iturin produced by the selected biocontrol strains which were the following: SZMC 27789: *Bacillus velezensis*, SZMC 27790: *Bacillus subtilis*, SZMC 27791:

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Bacillus altitudinis, SZMC 27792: *Bacillus paralicheniformis*. These measurements were performed from the same supernatant as the extracellular enzyme activity tests. These supernatant samples were prepared in PDB which contained lyophilized mycelia of *L. fungicola*, *H. perniciosus* or neither of them, thus we were able to compare the effect of these substances on the production of the aforementioned antibiotics. The antagonistic properties of these samples were also tested against two strains of *L. fungicola* and *H. perniciosus* each, as well as against single strains of *Trichoderma aggressivum* f. sp. *aggressivum*, *T. aggressivum* f. sp. *europaeum*, *Trichoderma pleuroticola*, *Trichoderma pleuroti* and *C. mycophilum*.

As a part of the ecophysiological characterization started last year, we also performed an experiment using PEG 6000 to identify the osmotic tolerance of the strains.

The *B. velezensis* and *B. subtilis* strains were tested in white button mushroom-growing experiments, where the bacteria were inoculated into the growing medium used in mushroom-cultivating facilities alongside with a strain of *L. fungicola*. This included experiments where the medium was inoculated with only the bacteria or the fungus, or neither of them.

An experiment was performed to test the growing and surviving abilities of the bacterial strains in the casing layer used in facilities growing white button mushroom. During the experiment the bacteria were inoculated into the sterilized casing layer material. Samples were collected daily and inoculated to yeast extract- and saccharose-containing media.

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Highly specific targeting of DNA methylation by the use of chemically modified oligonucleotides and evolved DNA methyltransferase

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DNA methylation is a fundamental part of the epigenetic toolkit of higher eukaryotes. C5-methylation, which occurs mainly in CG dinucleotides, has been widely studied and shown to play a role in shaping the gene expression patterns of these organisms. The role of DNA methylation in gene expression regulation is well established, but the underlying mechanism and the involvement of other epigenetic signals is not clear. Understanding the effects of DNA methylation can be adressed by using directed DNA methylation techniques to install methylcytosines at desired sites. In the available techniques, a C5-DNA methyltransferase is linked to a sequence-specific DNA-binding protein. An inherent consequence of the mechanism used by these systems is that they also methylate non-targeted sites at low frequency, which severely limits the utility of these methods.

To achieve much higher specificity than previous approaches, we aim to develop a targeted DNA methylation method based on a totally new principle. In the planned approach, we use a chemically modified oligonucleotide LNA (locked nucleic acid) or ENA (ethylene bridged nucleic acid) to promote DNA strand invasion at the targeted CG site. The oligonucleotide displaces one strand of the double-stranded DNA to form a heteroduplex structure, in which the targeted CG site on one strand is chemically modified. We plan to methylate the heteroduplex with a CG-specific DNA methyltransferase, which no longer has activity on the original native double-stranded DNA, but it is capable to methylate CG sites where one of the strands is chemically modified. We wish to create an enzyme with this altered specificity from two CG-specific bacterial DNA Mtases (M.SssI and M.MpeI). We have shown that methylation of a hemimethylated CG site by wild-type M.MpeI and M.SssI is almost completely blocked if the guanine in the non-substrate strand is replaced with ENA-**G** (5'-CG/5'-^{m}C**G**). These results show that M.SssI and M.MpeI are sensitive to ENA-**G** modification in the substrate site, and suggest that creation of mutant MTases displaying reversed substrate preference, *i.e.* specific for ENA-**G** substituted sites, could be possible.

For DNA strand invasion, we synthesized LNA (locked nucleic acid) and ENA (ethylene bridged nucleic acid) modified oligonucleotides which bind to their complementary sequences with high affinity. Due to their high affinity, they are able to invade into double-stranded DNA forming a local heteroduplex. We demonstrated strand invasion by the modified oligonucleotides, but the process was too slow to be applied in biological systems. We were able to increase the speed of strand invasion by forming an R-Loop in the close proximity of the target sequence by using CRISPR-dCas9. Using this ability of dCas9, we can achieve a biologically relevant DNA strand invasion speed.

To generate a methyltransferase specific for CG sites in ENA-DNA heteroduplex, we use structure-guided mutagenesis of

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M.MpeI and M.SssI to perturb the contacts between the enzyme and the sugar-phosphate backbone of the DNA. The mutant enzymes are purified by affinity chromatography and their substrate preference is characterized by *in vitro* radioactive MTase assay using [methyl-^{3}H]-labeled S-adenosylmethionine and double stranded oligonucleotides containing CG sites with or without ENA-**G** replacement in one strand.

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Analysis of HMGB proteins

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Beside linker histones (H1 and H5), B-type high mobility group box domain proteins (HMGB) are also important architectural components of chromatin. These proteins can bind to linker DNA and induce or repress gene expression. They can interact with both DNA and protein components of chromatin through their high mobility group box domains. Three architectural HMGB proteins, namely HmbA, HmbB and HmbC, were identified in *Aspergillus nidulans*, which of them the function of HmbB had been studied yet in details.

The *hmbBΔ* mutant is viable but displays severe fitness loss due to the drastic reduction in the viability of conidiospores and ascospores. Besides its role in spore viability, HmbB is also involved in diverse biological processes such as sugar metabolism during conidia germination, maintenance of the intracellular redox-balance or sterigmatocystin production. During my research, I am analysing the role of the HmbA protein. The *hmbAΔ* mutant strain has some obvious fenotype like forming small and compact colonies, slower hypha elongation compared to the wild type and empty, ascospore-free cleistothecia. In addition, the deletion of *hmbA* results in altered chitin deposition at the hyphal tips, production of thin hyphae and an abnormal mode of hypha elongation. We analysed this phenomenon and found out that overexpression of the endochitinase coding *chiA* gene re-establishes the hyphal elongation rate to normal (the colony size become wild-type-like), improves the hypha morphology and restores the diffuse distribution of chitin in the sub-apical area. Recently we focused on the study of the role of *chiA* gene and another putative endochitinase, AN11059 gene in *chiAΔ*, *11059Δ* and *chiAΔ*, *AN11059Δ* double mutant strains.

Based on previous study, we know that deletion of the unique gene encoding a typical linker histone H1 (*hhoA*) has no apparent phenotype in *Aspergillus nidulans*. Do the HMGB proteins complement the H1 histone functions in a *hhoAΔ* strain? In order to ask this question, we aimed to create a *hmbAΔ,BΔ,CΔ* and *hhoAΔ* quadruple deletion mutant strain and assess the role of H1 histone by comparing the phenotype of the quadruple mutant to a *hmbAΔ,BΔ,CΔ* triple mutant.

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Investigation of the effect of *Candida*-derived extracellular vesicles and candidalysin to the progression of oral squamous cell carcinoma

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Oral cancer is a serious health issue worldwide. Among the member states of the European Union, Central Europe, including Hungary has the highest incidence of oral cavity tumors. 90% of the mentioned tumor type is oral squamous cell carcinoma (OSCC). In the case of OSCC, oral candidiasis often develops due to the applied tumor therapy and the changed immunological microenvironment. A previous study of our laboratory has shown that, in case of OSCC, the number and diversity of colonizing yeasts in the oral cavity increases significantly, compared to healthy individuals. Furthermore, the presence of the causative agent of oral candidiasis, *Candida albicans* contributes to tumor progression by increasing the amount of oncometabolites produced by tumor cells, the activity of secreted matrix metalloproteinases (MMPs) and signaling pathways involved in tumor progression *in vitro* and *in vivo*. An important virulence factor of *C. albicans* is candidalysin, which is a pore-forming

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toxin encoded by the ECE1 gene. Because of the pore forming effect of this toxin, it can cause the damage of the epithelial cells and can increase the activity various signaling pathways which can role in the initiation and the progression of OSCC. The interaction between fungi and tumor cells can occur directly through different receptors, which we have previously examined, and indirectly through the molecules and particles secreted by them. During our work, we aimed to investigate the effect of candidalysin and extracellular vesicles (EVs) produced by *Candida* cells to the progression of OSCC.

During our experiments, we used EVs isolated from *Candida albicans* culture and ECE1 deletion mutant *C. albicans* strains that do not produce candidalysin to treat HSC-2 human OSCC cells. After the treatment we investigated the activity and migration of MMPs secreted by tumor cells, as well as the gene expression changes that occur as a result of the treatment after the fungus and EV treatment.

Our results showed that the presence of candidalysin is necessary to the *Candida* mediated tumor progression, because after the ECE1 deletion mutant strain treatment we could not detect any significant changes in the gene expression or migration of the cells or in the activity of secreted MMPs. The *Candida* derived EV treatment caused changes in the migration of the cells and in the activity of the secreted MMPs too. Thus, we hypothesize that *Candida* derived EVs can contain candidalysin.

Based on our results, we can conclude that EVs produced by *Candida* species and candidalysin also play an important role in tumor progression induced by the fungus.

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The loss of the accessory subunits of DNA polymerase epsilon POLE3 and POLE4 induces PARP inhibitor sensitivity

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ADP-ribosylation is a posttranslational modification that regulates fundamental cellular processes including but not limited to chromatin structure and DNA damage response. It is mediated by a family of enzymes called Poly(ADP-ribose) Polymerases (PARPs).

PARP inhibitors (PARPi) have emerged as a powerful tool in combating cancer. These selective anti-cancer drugs target and kill tumors with Breast Cancer Susceptibility protein (BRCA) deficiency sparing the healthy cells based on a phenomenon called "synthetic lethality", where two mutations or conditions combined kill the cell but not when they are alone. PARPi not only inhibits ADP-ribosylation but also increases PARP1 retention on sites of DNA damage causing a so-called "PARP trapping" phenomenon, a hallmark of PARPi sensitivity. These protein-DNA adducts can be transformed during replication into double-strand breaks (DSB). Cells that lack BRCA1, a crucial protein for the error-free homologous recombination (HR) pathway, are not able to resolve trapped PARP1 through HR leading to increased genomic instability and ultimately cytotoxicity. A model that explains the selectivity of PARPi towards BRCA-deficient tumors. However, the successful use of PARPi in the clinic is hindered by pre-existing and acquired resistance, such as the reactivation of homologous recombination. It is, therefore, crucial to identify mechanisms that re-sensitize such resistant cancer cells to PARPi.

In a genome-wide CRISPR knock-out screen, we identified DNA polymerase epsilon 3 (POLE3) and DNA polymerase epsilon 4 (POLE4) as potential inducers of sensitivity towards the FDA-approved PARPi, Olaparib. POLE3 and POLE4 are the two accessory subunits of the DNA polymerase epsilon holoenzyme, the polymerase mainly responsible for replicating the leading strand. While both accessory subunits are dispensable for replication, their yeast orthologs were implicated in intra-S-phase checkpoint signaling.

To validate the results of the screen, we generated knock-out cell lines for the two POLE subunits using CRISPR/Cas9. Indeed, the knock-out cells are hypersensitive towards various PARPi. Investigating PARP1 dynamics at sites of DNA damage revealed that deleting these subunits do not enhance PARP1 trapping ruling-out PARP1 being directly regulated by POLE 3/4. Olaparib treatment induces replication stress and G2 phase arrest in the POLE 3/4 knockouts, similarly to the effect in BRCA deficient cells. To determine the status of HR pathway when targeting POLE 3/4, we checked the ability of the knockout cells to mount foci of Rad51 protein, a critical step in HR responsible for homology search and strand invasion that leads to homology-directed repair, which is impaired in BRCA-deficient tumors. Surprisingly, POLE 3/4 deficient cells can efficiently

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recruit Rad51 upon Olaparib treatment suggesting an intact HR pathway even in the absence of these subunits. Significantly, the sensitivity of POLE 3/4 knockouts to Olaparib was enhanced when BRCA1 was missing indicating that both subunits act in a parallel pathway to BRCA1, which potentially offers a new therapeutic approach towards resistant tumors with reactivated HR pathway.

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