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POSTERS

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Abstracts submitted to the 48th FEBS Congress from 29th June to 3rd July 2024 and accepted by the Congress Scientific Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as three PDF files: Talks (Plenary Lectures, Symposia and Speed Talks), Posters and Posters Annex.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented.

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for a wide spectrum of ciliopathy outcomes, making difficult a definitive genetic diagnosis. Three morpholinos that specifically bind and block the expression of the ciliopathy related genes, *ahi1, tmem67* and *rpgripl1*, known to be associated with Joubert Syndrome, were injected in 2-cell stage zebrafish embryos and their development was investigated. Three days post fertilization, ahi1, tmem67 and rpgrip1l morphants showed hydrocephalic head, abnormal body curvature and heart bilateral symmetry. Whole mount immunofluorescence using anti acetylated tubulin antibody proved an impairment in cilia development at the level of olfactory placode ranging from few misshaped cilia in *tmem67* morphants to complete loss of cilia in *ahi1* and *rpgrip1l* morphants. To demonstrate the correlation of morphants phenotype to ciliopathies, a rescue experiment was carried out by simultaneously injecting in the embryos the morpholino and the human wild -ype mRNA. Morphological analyses confirmed a successful rescue of the phenotype. This approach will be exploited to investigate the effect of VUS on the penetrance and on the expressivity of the canonical recessive ciliopathy mutations. The possibility to easily generate zebrafish morphants and dissect the effect of specific mutations could help in solving diagnostic dilemmas also in other similarly "complex mendelian" disorders.

P-24-003
Application of type IV-A3 CRISPR-Cas system from *Klebsiella pneumoniae* to modulate gene expression in mammalian cells

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Identification of CRISPR-Cas tools as precise genome editors opened new avenues for the treatment of various human diseases. However, conventional CRISPR techniques rely on the generation of site-specific DNA double-strand breaks (DSB), which inevitably come with risks, from unspecific mutations at the site of the break to large-scale chromosome rearrangement; therefore, alternative approaches to DNA cutting and editing are required. Recently described type IV-A3 CRISPR-Cas system from *Klebsiella pneumoniae* do not require DSB to target specific gene expression, as this system, composed of a multi-subunit effector complex and a CasDinG helicase, uses a nuclease-independent transcriptional interference pathway to suppress gene expression. While type IV-A3 CRISPR-Cas system has gene repression activity in bacterial cells, its application in mammalian cells has not yet been established. In this study, we expressed the type IV-A3 system along with non-targeting RNA in human embryonic kidney HEK293T cells. To optimize the expression, we used immunofluorescent labeling to quantify the proportion of cells expressing type IV-A3 complex proteins and evaluated DNA interference at single-cell level. To apply type IV-A3 CRISPR system for transcription interference in HEK293T cells we targeted highly conserved PPIB gene, which is used for the benchmarking of the modulation of gene expression. For PPIB expression interference, we used ten RNA guides for both coding and non-coding regions of PPIB gene. We evaluated transcription interference by quantitative real-time PCR. In parallel, we have evaluated transfection efficiency by immunofluorescence microscopy. Our study revealed that type IV-A3 CRISPR system can be used for targeted genome modulation in HEK293T cells. Application of this CRISPR system in mammalian cells offers the opportunity to expand the CRISPR toolbox with potentially safer techniques that elicit targeted changes to the genome without the introduction of a DSBs.

P-24-004
CRISPR/Cas9-mediated UBC knockout in gastric cancer cell lines

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Gastric cancer (GC) is one of the most common and lethal cancers. Alterations in the ubiquitin proteasome system (UPS) play key roles in the carcinogenetic process. Hence, the ubiquitin pathway is considered a promising target for therapeutic interventions. Upregulation of polyubiquitin genes *UBB* and *UBC* is observed in various cancers, with studies demonstrating that silencing *UBB* reduces proliferation rates in multiple cancer types, while knockdown of *UBB* and *UBC* inhibits lung cancer cell growth both *in vitro* and *in vivo*[1]. Our investigations in GC cell lines show that siRNA-mediated knockdown of *UBB* and *UBC* induces apoptosis predominantly in primary cells[2]. The CRISPR/Cas9 system has recently used to deplete ubiquitin pools by knockout of *UBB* and *UBC*[3] . Our aim was to knockout the unique coding exon of the *UBC* gene using the CRISPR/Cas9 system in primary and metastatic GC cell lines. Dual guide RNAs (gRNAs) targeting *UBC* were cloned into the Cas9 expression vector PX459, facilitated precise excision of the *UBC* coding exon, confirmed through PCR amplification of genomic DNA. Initial trials in HEK293T demonstrated successful *UBC* knockout. Western blot analysis confirmed a decrease in total ubiquitin levels in *UBC*-KO cells. Further, we conducted CRISPR/Cas9-driven *UBC* KO in GC cell lines, observing varying responses in ubiquitin pool depletion between the two GC cell lines, resulting in reduced *UBC* mRNA expression and diminished total ubiquitin levels. Clonal expansion of transfected GC cells yielded *UBC*-knockout cell lines for further analyses, focusing on elucidating effects on other ubiquitin gene expression, proliferation rates, cell cycle distribution, and migration capabilities. Targeting the ubiquitin pathway via gene knockout shows promise as a novel therapeutic strategy in GC, with further understanding of its role in cancer biology potentially leading to new therapeutic avenues. References: [1]Tang, Y. et al. (2015) Sci Rep **5**, 9476 (2015). https://doi.org/10.1038/srep09476 [2]Scarpa, E.S. et al (2020) Int J Mol Sci 21(15), 5435 https://doi.org/10.3390/ijms21155435 [3]Park C.W. et al. (2020) Cell Biochem Biophys 78(3):321-329 doi: 10.1007/s12013-020-00933-2