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Regulatory functions of the upstream open reading frame in mRNA of the *MCRS1* human gene

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Motivation and Aim: Small open reading frames (sORFs) are characterized by a short length (below 100 codons) compared to main open reading frames (mORF) encoding functional proteins. sORFs were considered nonfunctional initially, but later studies showed that many of them are involved in important physiological processes. sORFs are located in many types of RNA, both coding (mRNA) and non-coding, but most currently known sORFs are located in the 5' untranslated regions (5' UTR) of coding transcripts (upstream ORFs, uORF). Moreover, upstream ORFs may encode functional microproteins or play regulatory roles [1]. In particular, they may repress the translation of the downstream main ORFs or contribute to their translation in particular conditions [2]. The CRISPR-Cas9 screening previously performed in the laboratory has discovered that the disruption of uORFs in several human genes affects viability of near haploid HAP1 human cell line. One of the identified hits is located in *MCRS1* gene. Subsequent analysis showed that the *MCRS1* gene mRNA contains three small open reading frames in the 5' UTR, varying in length (sORF#1, sORF#2, sORF#3). The aim of the research is to validate sORFs translation and to study their biological function.
Methods and Algorithms: We obtained reporter constructions with the *MCRS1* 5' UTR to validate the translation of its sORFs and to find out the role of the longest sORF#1 in mORF translation. We have done genome editing with CRISPR-Cas9 for endogenous tagging of the sORF with HiBiT-tag to prove the existence of the micropeptide *in vivo*.
Results: We showed that the sORF#1 of the *MCRS1* gene is translated. sORF#1 encodes a 46 amino acid micropeptide, which is conserved among mammals. The presence of this micropeptide was proven *in vivo* using endogenous labeling and subsequent detection of the labeled micropeptide. Mutations in *MCRS1* sORF#1 introduced by CRISPR-Cas9 decrease cell viability. Furthermore, *MCRS1* sORF#1 represses the translation of the main ORF. The overexpression of the 5' UTR of *MCRS1* gene with HiBiT-tag insertion demonstrated that the shorter sORF#2 can also be translated. Using the dual luciferase reporter with cloned 5' UTR of the *MCRS1* gene, we found out that the second start codon of the mORF is involved in translation initiation; *MCRS1* translation predominantly begins with it. The first start codon of mORF takes almost no part in the initiation of *MCRS1* translation. Elongation of the sORF#1 does not affect the efficiency of translation of the mORF. sORF#1 shortening activates translation of the mORF. At the same time, we demonstrated that the *MCRS1* mRNA is not a subject to

regulation through nonsense-mediated decay as shown by the translation arrest using cycloheximide.

Conclusion: In conclusion, our study shows that sORF#1 regulates the translation of mORF of the *MCRS1* gene, and sORF#1 functioning is necessary for the cell viability. However, the detailed mechanism of the entire process remains unclear. Further research will elucidate the mechanisms of translation regulation by *MCRS1* sORF#1 and investigate the functions of the encoded microprotein to provide a complete picture of sORF functioning and open up new prospects for further research.

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