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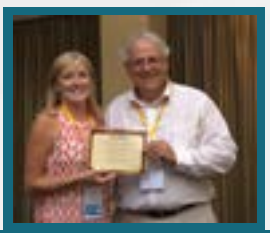


Posters

CRISPR/Cas9 based LRP2 gene knockout model applied in evaluation of potential LRP2 substrates

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Low-Density Lipoprotein Receptor-Related Protein 2 (LRP2, megalin) belongs to the LDL receptor family localized on the apical surface of several different epithelial cells. The receptor is responsible for internalization of various ligands including aminoglycoside antibiotics, hormones and their carriers, nutrients etc. The common feature for all known ligands is their high molecular weight.

Human cell lines originally expressing LRP2 – HK2 (proximal tubular cell line) and JEG-3 (human placental choriocarcinoma cell line) were used for gene modification to improve the characterization of possible LRP2 substrates. The knockout of LRP2 gene was achieved using CRISPR/Cas9 method. sgRNA sequences were specifically designed to target crucial sites for regulation of function and trafficking ligands (NPMY motif), phosphorylation (PPPSP motif) or transmembrane domain of LRP2. The modification was verified by two different methods based on various response of unmodified and newly modified cells to well-known LRP2 ligands aminoglycoside antibiotic gentamicin and FITC-albumin. The first method monitors increased viability of transfected cells treated with cytotoxic doses of gentamicin. The second method detects lower amount of accumulated FITC-albumin in modified cells. FACS (fluorescent activated cell sorting) was used to secure separation of genetically modified cells exerting low activity of megalin from unmodified cells. After confirmation of lower LRP2 expression in sorted cells by qRT-PCR and control methods, developed cells can be considered as a suitable model for further testing of potential LRP2 ligands.

The first tested substance was radiolabeled VEGF-A N-terminal helix-derived 15 amino acid peptide with binding and inhibitory potency to VEGF (Vascular Endothelial Growth Factor) receptors. We confirmed the 15-mer as LRP2 ligand based on the accumulation studies results. Further testing of more potential substances is planned in near future.

sgRNA designed to target

- NPMY - motif important for internalization and function
- PPPSP - phosphorylation site
- TMD - transmembrane domain

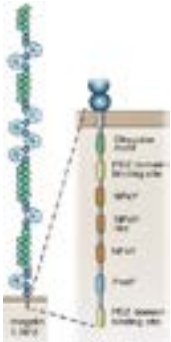

Fig1: Structure of LRP2 and localization of crucial sites for regulation of function (Willnow et al., 2017)

Biography

Anna Durinova is a PhD. student at Charles University, Faculty of Pharmacy in Hradec Kralove where she works as a member of Pharmacology and Toxicology department. Her area of interest is in the field of molecular pharmacology focused on use of molecular biology techniques, such as CRISPR/Cas 9 gene editing, flow cytometry, FACS and qRT-PCR method. Within her study she has gained expertise with radiolabeled substances used in accumulation studies.

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Maryam Sadat Moosavi, J Biotechnol Biomater 2022, Volume 12

The amount of iron can control the level of mitochondrial activity and subsequently the synthesis and expression of genes

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Iron plays an important role in electron transfer, cellular respiration, differentiation and regulation of gene transcription, DNA synthesis and repair and role in supporting the transcription of key genes required for cell growth and function [such as: nitric oxide synthase, protein kinase c-beta, p21(CIP1/WAF1)] Mitochondria are the main centers of iron accumulation and utilization. It can be concluded that it is the mitochondria that regulates the iron, but another conclusion is that it is the iron that controls the mitochondria, how much of the necessary enzymes to be made or how much of the genes involved in cellular activities are expressed. It can be concluded that the iron controls the mitochondria, how much of the necessary enzymes to be made or how much of the genes involved in cellular activities are expressed. This conclusion can be because it is stored in the form of ferritin in parts with high activity and gene expression and high differentiation, i.e. intestinal mucosa, liver, spleen, kidney, and bone marrow, or in nerve cells, red blood cells, and macrophages Entrusts export iron. considering that the regulation of iron metabolism is responsible for intestinal absorption of iron and stress has the greatest effect on stomach acid and digestive system and liver activity, with the increase of stomach acid, the environment becomes more suitable for dissolving iron. On the other hand, hepcidin of the liver, which is a peptide that controls the level of iron in the blood, by binding to ferroportin, prevents the release of iron from the cells. Therefore, an imbalance occurs because the iron entering the cells is high, but the outgoing iron is low. This can be the reason for the accumulation of iron in cancer cells. By iron increasing, all the above things, such as DNA synthesis and expression, and energy increase.

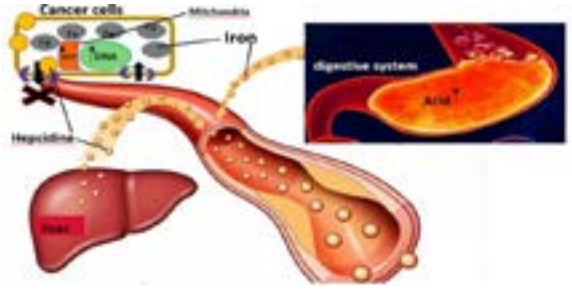

***Figure 1**: Many vital genes, including genes controlling cell division, respiration, DNA synthesis and repair, and regulation of gene expression, including oncogenes and tumor suppressor genes, are controlled by iron. Mitochondria, which is the control center of transcription, differentiation, respiration and energy production and cell death, the most iron is located in mitochondria. In the face of stress, when stomach acid, which is the most important factor in iron absorption, increases, we also see changes in the production and release of hepcidin in our liver due to stress. An increase in iron on the one hand and a decrease in the release of iron from the cell traps iron, and subsequently all the functions of iron become uncontrolled and become cancerous.*

Biography

Maryam Mousavi received her bachelor's degree in genetics from the University of Isfahan. During this time, she learned some laboratory work. she completed her master's degree at Ashrafi University of Isfahani with the same professors of Isfahan University. She wrote several articles on different topics and learned laboratory work in a more specialized way. By reading many articles, she try to find answers to her questions and present them in the form of articles for further follow-up by people with more information and capabilities.

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