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

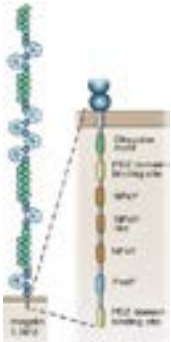

Fig1: Structure of LRP2 and localization of crucial sites for regulation of function (Willnow et al., 2017)

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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