One of the most common features of living organisms is their ability to perform metabolic processes, which involve the transformation of matter and energy through various chemical reactions. These reactions are arranged in pathways that are mediated by enzymes, specialized proteins that can speed up specific chemical reactions. Most of the metabolic pathways in humans are well-known, but there are still some enzymes that have unknown functions and may participate in new metabolic pathways in humans. For instance, some studies have shown that mammals have a capacity to break down L-fucose, a rare sugar that has many biological roles, such as determining blood types and modulating immune responses. However, the molecular identity and biological significance of this pathway have not been clarified. In our laboratory, we have recently discovered a mammalian gene that codes for 2-keto-3-deoxy-L-fuconate dehydrogenase, an enzyme of the L-fucose degradation pathway. Interestingly, it has been reported that the absence of this gene in mice causes severe problems in the regulation of intracellular iron levels. However, the exact biochemical mechanisms behind this metabolic disorder are largely unknown.

The aim of this project is to investigate the role of 2-keto-3-deoxy-L-fuconate dehydrogenase, an enzyme involved in the metabolism of L-fucose, in iron homeostasis. To achieve this goal, we will:

1) produce ‘synthetic’, recombinant human and mouse 2-keto-3-deoxy-L-fuconate dehydrogenase and perform biochemical and functional characterization of these enzymes, including their substrate specificity, kinetic parameters, and regulation,
2) generate mice cells without 2-keto-3-deoxy-L-fuconate dehydrogenase activity and employ them to disclose the metabolic consequences of the enzyme loss for the cells, such as changes in L-fucose and heme metabolism, iron levels, and cell and mitochondria viability,
3) identify the biochemical mechanism(s) responsible for the excessive accumulation of iron in the cytoplasm of 2-keto-3-deoxy-L-fuconate dehydrogenase-deficient cells, by exploring the possible involvement of specific metabolic blocks, endogenous chelators, and protein fucosylation.

Recombinant enzymes will be expressed in *Escherichia coli*, purified by affinity chromatography, and tested for their catalytic activity using a spectrophotometric method. The chemical identity of the enzymatic products will be verified by ion chromatography and mass spectrometry. The CRISPR / Cas9 technique will be applied to create mouse cell lines that lack 2-keto-3-deoxy-L-fuconate dehydrogenase activity, and the impact of the disruption of L-fucose degradation pathway on iron homeostasis will be assessed by radiochemical and chromatographic techniques.

The successful completion of this project will result in a comprehensive biochemical and functional characterization of 2-keto-3-deoxy-L-fuconate dehydrogenase. By elucidating the biochemical properties and functions of this enzyme, we will gain insight into the biochemical basis of the iron homeostasis disturbances that occur in 2-keto-3-deoxy-L-fuconate dehydrogenase-deficient cells. Furthermore, we anticipate that the results of this project will enable the discovery of new human diseases related to L-fucose breakdown.