Many neurodevelopmental diseases are characterized by mitochondrial dysfunction -organelles responsible for cellular respiration, but the nature of molecular mechanisms underlying this dysfunction and whether it also depends on the mitochondrial biogenesis, has not been clarified so far. We know, however, that in the early stages of human development, pluripotent stem cells, from which the whole organism including the Central Nervous System is formed, undergo a process of changing respiration from glycolytic, not depending on mitochondria, to aerobic, in which both, the number of mitochondria, as well as their structural/functional maturity, are important.

The presented project entitled: "Deciphering the effect of PGC-1α on mitochondrial biogenesis and neural differentiation during early development of human cerebral organoids" attempts to explain the role of mitochondrial biogenesis during early human brain development using, as a research model, cerebral organoids, obtained *in vitro* from human induced pluripotent stem cells (iPSC). Since PGC-1α is transcriptional co-activator and positive master regulator of mitochondrial biogenesis, we want to verify whether manipulation with expression of PGC-1α will influence neural type content in developing cerebral organoids. Mitochondrial biogenesis will be simulated and trace developmental changes induced by this stimulation in the cerebral organoids at various stages of their differentiation. In addition we will try to unravel molecular interrelation between pathways involved in mitochondrial biogenesis and differentiation of neuronal cells. Tracing the changes induced by stimulation of mitochondrial biogenesis on the cellular level in living cells will be possible due to the presence of a fluorescent protein in the system, which starts to glow only when the determinant of mitochondrial biogenesis - PGC-1α protein - is expressed. Such an effect can be achieved using genetic engineering methods, which allow to generate the so-called the "reporter line" of pluripotent stem cells, which will serve us further to obtain *in vitro* culture of human brain organoids at various stages of development: embryonic bodies, neurospheres and cerebral organoids (i.e., brain-cortex-like structures). In addition we will design and use molecular "optogenetic" system which allow to "switch on and off" by light activity od desired gene. The plan of our project is based on the application of three ground-breaking achievements of recent years in the field of stem cells, molecular biology and tissue engineering, these are:

1) obtaining human induced pluripotent stem cells (iPSC), which are an alternative to human embryonic stem cell research, because they have similar properties and are not ethically controversies. What's more, every cell of the body can be "reprogrammed" into an iPSC cell, creating an infinite source of cells for research and therapy using patient's own cells – known as personalized medicine;
2) developing of the "genome editing" method (CRISPR/CAS9), which allows to precisely and safely put in ("Knock-in") or cut out ("Knock-out") DNA sequences;
3) developing of optogenetic molecular system "switch on and off" by light activity of desired gene.
4) obtaining organoids in *in vitro* culture, structures reminiscent of human organs, which reflect the early embryonic development of these organs. Brain organoids, derived from human stem cells, are an innovative, convenient and alternative model system of early neural development that responds to pharmacological and genetic manipulation, which is not possible in human applications.

The reporter cell line "IPS-*PPRGCA1*-DsRed2-Mito-7" and derived from this cell line human brain organoids, will be obtained *in vitro* due to the application of mentioned above emerging technologies. In addition to tracing expected PGC-1α gene expression changes with associated fluorescent protein (possibility for observation in living cells), differences in gene expression on RNA level between organoids at different stages of development in control and experiment we will be determined by genome wide sequencing supported by qRT-PCR for the selected genes.

The project will verify the hypotheses concerning the impact of stimulation of mitochondrial biogenesis on neural differentiation and whether this cellular response is linked to the activation activating of PGC-1α signaling (the main regulator of mitochondrial biogenesis) and nuclear FGFR1 signaling pathway (important for early neural development and also involved in the pathology of schizophrenia). We will also determine whether the induction of mitochondrial biogenesis is possible at all tested developmental stages and we are predicting, that the cellular response will be developmental stage dependent.

The results obtained will not only define the role of mitochondrial biogenesis in the model of brain organoids and explain some of the molecular mechanisms underlying neural cell fate decisions, but will also approximate the 'window of sensitivity' to the stage of early neural development for potential therapeutic agents of neurodevelopmental disorders linked to mitochondria