The aim of this project is the investigation of DNA repair processes in human induced pluripotent stem cells (iPSC) during embryotoxic and teratogenic response to methylmercury chloride and low oxygen conditions.

The discovery of iPS cells aroused many emotions. The established method of pluripotent induction has allowed to obtain iPSC possessing similar potential to human embryonic stem cells. A change of somatic cells phenotype allowed to introduce the transcription factors such as: OCT3/4, SOX2, NANOG, KLF4 and c-MYC that brought about desired genetic, epigenetic and metabolic changes. Microarray method helped to show a considerable similarity on the molecular level of embryonic stem cells to cells obtained by reprogramming.

The characteristic feature of pluripotent cells istheir ability to differentiate into any type of the body. In functional tests it proved by the capability of forming embryoid bodies (EBs), generation of chimerical embryo after injecting pluripotent cells into blastocysts and creating teratoma after injecting into immonocompetent mouse. The production of induce pluripotent stem cells was a breakthrough towards the development of regenerative medicine, since it opens possibility for personalized clinical treatments.

The first human embryonic stem cells were obtained in 90s from: supernumerary embryos coming from in vitro fertilization. In Poland implemented law has been very restrict and forbids the work on any embryonic stem cells. The legislation forbids from obtaining and all studies on human embryonic stem cells thus there is a lack of human model towards study over embryotoxicity and teratognicity in vitro. Induce pluripotent stem cells thanks to the similarity to embryonic stem cells constitute the ethical model of human embryo. The studies on reprogramming allow to comprehend better the molecular basics of embryonic development without ethical controversies. Such studies are of high importance for better understanding of molecular mechanisms of early embryo development and may have predictive value for estimation of the adverse influence of environmental factors including those that influence development of human Central Nervous System

iPS cells are already used in creating the models of in vitro diseases and constitute a valuable model to test the new drugs in basic studies. The database ClinincalTrialis.gov provides information on 10 ongoing clinical trials using human iPSC, however most of them are obtaining and characterizing in vitro of therapeutically competent cell population and only in two indicated trials human iPS cells are given to the patients. Indeed human iPSC due to the way of their obtaining they possess a big advantage over other stem cells and will be used in many clinical trials towards burdensome of different human diseases.

The basic disadvantage for the security of potential therapy of pluripotent stem cells, (both iPSC and ESC) is their ability to form teratoma and their low genetic stability. The adverse effect of teratoma formation can be resolved by transplantation of the predifferentiated iPS cells, however the problem of genetic instability at different microenvironmental conditions require more basic science investigations, that have not been conducted yet.

The first iPS cells were introduced by means of retroviruses but quickly it was found that they create malignant tumors in immunocompetent mouse due to insertional mutagenesis. The two proto-oncogenes: c-MYC and KLF4 as well as p53 gene silencing used in reprogramming play an important function in tumor development.

Today the great improvement in the derivation of human iPS cells using “safe” non-integrative methods and excluding protooncogens from the reprogramming cocktail have been made. However due to the high therapeutic application potential of iPS cells and even the start of clinical trials, the problems regarding their genetic instability need to be deeper explored by investigating the mechanisms of DNA repair in these cells. The present study include methyl mercury chloride and low oxygen level to induce DNA damage in such studies

Growing data indicate that low oxygen level conditions influence the process of differentiation of iPS cells into neural lineages. However the effect of lowering the oxygen level from ambient (21%) to physiological (5%) level in iPSC culture has to be in connection to DNA damage and repair needs to be explored in connection with the safety issues. Low oxygen level promotes differentiation of iPS cells to obtain desirable phenotype of, but on the other hand, in the case of tumor cells cause a repression of DNA repair genes.

Methylemercury chloride is widely investigated toxic compound classified as a developmental neurotoxin, targeting especially Central Nervous System during its development. Importantly it generate different type of DNA damage giving the experimental opportunity to examine the process of DNA damage and repair. Implementation of the project: “Embriotoxicity and teratogenicity as a result of the response to DNA damage in iPS cells: methylemercury chloride and low oxygen level effect” will allow to establish gen expression of DNA repair in “the ethical model of human embryo” in oxygen environment related to the conditions of its development. In human ESC model 71 teratogenic substances were found to have an adverse impact on the development of embryo. Human iPS cells give the opportunity to serve as a model for such studies without damaging of human embryo. In addition the considerable restriction of the research on animals as a result of the legislation and the promotion of the development of alternative models in toxicology have been introduced. Thus human iPS cells modeling embryonic development provide a very important tool that allows to obtain an unique knowledge on of DNA repair at early developmental phase without using human embryonic stem cells.

Since human iPS cells were obtained in 2007, they are just started to be explored in the field of in vitro developmental toxicology. Its implementation is especially important in the countries in which the research on human embryonic stem cells is forbidden by law. Teratogencity and embryotoxicity of methylemercury chloride proved by in vivo and in vitro on ESC cells need to be also approved in iPSC to increase the credibility of “the ethical model of human embryo”.

The primary researches that will be conducted in the project include: the evaluation of cytotoxicity, genotoxicity, apoptosis, analysis of DNA damage as well as the expression of DNA repair and hypoxia linked genes measured with PCR method at the real time.

The obtained data will also add desired information on the role of methylemercury chloride in disruption of the expression of DNA repair genes in the conditions of low, physiological oxygen tension. Such knowledge is very important to understand the role of methylemercury chloride in pathogenesis of neurodegenerative diseases.