Background. Human genome, nuclear and mitochondrial, is exposed to various endogenous and exogenous damaging factors. DNA suffers various types of damage, including breaks, and undergoes continuous repair. When the level of damage increases, the likelihood of unsuccessful or erroneous repair increases as well. Errors in DNA damage may lead to changes in genetic code resulting in cell death, but also to chromosome structural aberrations and cancer, and to an increase of the levels of cytosolic DNA (DNA fragments found outside of the cell nucleus and mitochondria), which is thought to promote autoimmune disorders. DNA damage is particularly dangerous to dividing cells, including stem and bone marrow cells, although DNA lesions are also dangerous to non-dividing ones, like neurons. The number of endogenous unrepaired DNA breaks is believed to increase with the age of the organism, however the available information is based on indirect measurements, and the exact levels of endogenous damage in various human tissues remains to be investigated.

Hypothesis. The number of endogenous DNA breaks in the cell nucleus increases with the age of a mammalian organism and is strongly dependent on the type of tissue. The age-related increase of the number of endogenous DNA lesions results in an increase of the levels of cytosolic DNA.

Aims. The aims of the planned research include (i) quantifying endogenous DNA single- and double-strand breaks in nuclei of cells of various mouse and human tissues as a function of age of an organism, (ii) quantifying endogenous single- and double-strand DNA breaks residing in eu- and heterochromatin of cells from various tissues, and in DNA replicating vs. non-replicating cells, (iii) quantifying single- and double-strand breaks in mitochondrial DNA in tissues of mice and humans of various ages, (iv) quantifying cytosolic DNA fragments in cells of various tissues, and relating them to the age of an organism, (v) assessing the relationship between the levels of age-related endogenous DNA damage and the levels of cytosolic DNA in various tissues, and (vi) assessing endogenous damage and cytosolic DNA in human cells grown under laboratory conditions which are propagated to be used as skin or connective tissue grafts.

Methods. Two new advanced, highly sensitive and specific techniques of direct detection of single- and double-strand DNA breaks (sSTRIDE and dSTRIDE), combined with super-resolution and high sensitivity fluorescence imaging (dSTORM, widefield microscopy with EMCCD detectors) open new possibilities in detecting and quantifying endogenous damage, and fragments of cytosolic DNA, in various human tissues, and organisms of different ages. These new approaches can also be adapted for assessing the quality of DNA in human keratinocytes and chondrocytes grown under laboratory conditions and intended for grafts.

Significance. The planned research will provide information about the levels of endogenous DNA breaks in various mouse and human tissues and will allow analysis of correlations between the levels of endogenous DNA damage and the amount of cytosolic DNA in various tissues. This work will also open a possibility of using STRIDE for verifying the quality of DNA in laboratory grown cells used for skin and connective tissue grafts.

Preliminary data. In preliminary experiments single- and double-strand DNA breaks as well as cytosolic DNA fragments were successfully detected and quantified in cryosections of various mouse tissues, including striated muscle, liver and kidney.