Ageing of the organism is associated with a decrease in the functioning of tissues and organs, and with an increased incidence of age-related diseases. Cellular senescence is inextricably linked to the aging of the whole organism. In the senescent cell there are a number of changes related to the metabolism, gene expression and the structure of cellular organelles and macromolecules. These changes are also related to the nucleus and chromatin structure. They concern not only an increase in the extent of DNA damage, which accumulates during senescence/aging, but also the composition of proteins associated with the proper function of the nuclear envelope (change in the ratio between lamin and progerin in favor of the latter). The changes also comprise reduction of heterochromatin content, increase in euchromatin and loss of histones that form nucleosomes. Such changes result in impaired structure and function of chromatin and in consequence promote DNA damage and alter the expression of genes. One of the main reasons of senescence-associated changes of the chromatin are epigenetic modifications of both DNA and core histones. Senescence is accompanied by a decrease in the methylation of histone H3 at lysine 9 (H3K9me3) and in the level of HP1 (heterochromatin protein 1), which upon binding to H3K9me3, promote the formation of heterochromatin.

Despite the fact, that the primary cause of senescence can differ (telomere erosion during replicative senescence or stress caused premature senescence), senescent cells express some universal markers which are characteristic for all types of senescence. However, some differences in cell phenotype can be specific for a particular type of senescence as even such universal process like senescence can proceed differently in cells derived from various tissues (cell-type exclusive senescent phenotype - CESP). In particular little is known about the differences connected with the type of senescence and cell type in the case of chromatin. Additionally, the existing data concerning chromatin changes during senescence are confusing. On the one hand the loss of histones and deheterochromatinization takes place during senescence but, on the other hand, in senescent cells foci of condensed chromatin, named SAHF (senescence associated heterochromatin foci), can be observed. Our preliminary results showed that in the case of vascular smooth muscle cells (VSMC) the changes in histone H3 modifications differ dependently on the type of senescence. During replicative senescence a reduction in the levels of H3K9me3 and HP1 alpha occurred and changes in the spatial organization of HP1 alpha were observed. In the case of premature senescence a decrease neither in H3K9me3 nor HP1 alpha were detected and only changes in the organization of HP1 alpha were observed. Therefore, **the aim of the project is to analyze changes in the chromatin structure during vascular smooth muscle cells senescence, with particular emphasis on the role of HP1 alpha in this process and modifications of histone H3. We postulate that in VSMC there are differences in modifications of histone H3 during replicative and premature senescence and that deheterochromatinization is characteristic only for replicative senescence. We would like to identify chromosomal regions which differ in histone H3 modifications in both types of senescence and to determine whether these changes have an impact on the expression of genes located in these regions. We would like to compare if the same changes take place during *in vitro* and *in vivo* senescence.**

We plan to analyze modifications of histone H3 (H3K9ac, H3K9me3, H3K27me3 and H3K4me3) and the level of HP1 alpha in different types of senescence (ChIP-seq, immunocytochemistry, Western blot analysis) and to analyze the chromatin structure (digestion by micrococcal nuclease, in situ nick translation, AFM). We intend to identify chromatin regions that display differences in the level of H3K9me3 and H3K4me3 (marker of euchromatin) in different types of senescence – according to the results obtained by ChIP-seq. Moreover, we would like to determine, if changes in H3K9 modifications lead to differences in gene expression (qPCR). We will analyze the selected modifications of histone H3 in cells derived from atherosclerotic plaques in order to compare senescence occurring *in vitro* and *in vivo*.

In the organism, cells senesce in both the replicative and premature manner. So far, there are no markers allowing to clearly distinguish replicative senescence from the premature one (except for analysis of the telomere length), especially when senescence occurs in *vivo*. Recognition of the differences in the histone H3 modifications in senescent cells is especially interesting and important in the case of cells building the vasculature. They are involved in the progression of atherosclerosis, a process in which the role of cellular senescence is particularly significant. Vascular cells are exposed to stress and are in a close contact with the blood compounds (drugs, harmful elements from the food). Knowledge about the differences in modifications of chromatin during replicative and premature senescence may be useful for the identification of markers specific for the particular type of senescence. Such markers could be used in the future diagnostics to predict the risk of atherosclerosis in advance, and in the study of the effect of compounds/drugs on cell senescence. Understanding the differences in chromatin modifications and how they affect gene expression can contribute to the elucidation of the role of cellular senescence in the process of atherosclerosis. The importance of the proposal is supported by the fact that the possibility of slowing down the replicative senescence is limited, while modifying or avoiding accelerated senescence seems to be possible