Aim of this project is to investigate possible influence of the cell cycle phase, amount of the SSBs and lack of XRCC1 protein on activation of Short-Patch (SP) and Long-Patch (LP) single-strand breaks repair pathways. DNA of living cells is constantly exposed to different types of damage of which most popular are single-strand breaks (SSBs). These can occur due to attack of intracellular metabolites, reactive oxygen species and many more. Potentially harmless SSBs, when unrepaired, can be converted into double-strand breaks (DSBs) leading to the serious mutations, carcinogenesis or even cell death. That's why it is very important to understand how repair mechanisms of SSBs work and what can affects their proper functioning. There are two SSBs repair pathways – SP and LP that can effectively deal with this lesions. One can find information in literature about factors influencing activation of SP and LP repair pathways such as ATP concentration, cell differentiation or interaction between repair factors. There is only a little information about impact of the cell cycle phase, amount of SSBs and lack of key factor – XRCC1 on the SSBR processes. Experiments carried out so far to study influence of mentioned above factors were based on biochemical approaches (COMET Assay, alcalic elution). Damage was induced by UV, visible light and X irradiation, very often combined with photosensitizers, chemical agents such as MMS or H_2O_2 (big number of damage of different types). In addition cells were usually synchronised in the cell cycle what further generated cellular stress and damage. This kind of approaches make results difficult to interpret and conditions are far from physiological what rarely occur in living organisms. We have created new method for generating SSBs with focused beam of visible light in confocal microscope. It allows to damage any region of interest within nucleus. We can manipulate the illumination time and total energy delivered to the cell to induce damage of certain type (SSBs without DSBs and oxidative damage). Our method allows to investigate repair processes of very small amounts of SSBs in single cell without use of other damaging agents. In our experiments we plan to induce SSBs in HeLa 21-4 cells expressing proteins present in different stages of SSBs repair processes (PCNA, LIGI, LIGIII, XRCC1) where LIGI and LIGIII will be markers of LP and SP pathways, meanwhile PCNA due to it's pattern in replication will be used to distinguish replicating (Early, Middle, Late S phases) and non-replicating cells. Next step will be to induce growing number of SSBs in the living cells to check how many of these lesions can be simultaneously repaired. These experiments will provide information about the amount of proteins (XRCC1, PCNA, LIGI, LIGIII) participating in SSBs repair process at once. Last part of the project is meant to answer the question about repair processes under conditions of lack of XRCC1. We should get information about repair pathways being activated when XRCC1 is not available and possible conversion of SSBs to DSBs when cell is lacking it's SSBR key factor. To carry out this experiments, cells with XRCC1 partly and completely gene knockout will be used and treated with heat shock which generates SSBs in big populations of the cells. This project is expected to provide new important information about repair processes of SSBs, crucial to maintain genome stability and pass complete, undamaged information to next generations of the cells. I decided to work on this topic because of importance of these processes for proper functioning of every living organisms. Knowledge about these mechanisms can be used in development of new anticancer treatments and increase life quality of patients. New approach used in this project is perfect for testing existing hypothesis connected with repair of DNA SSBs in different stages of the cell cycle from new and alternative point of view.