

1. Introduction:

In Poland at 2012 the 17.000 new cases of breast cancer were diagnosed (1600 cases in Greater Poland). Thanks to early detection and well applied treatment over 90% of survival is observed. The complexity of cancer biology extort use local and systemic treatment approach. One of the common local methods used in treatment of cancer is radiotherapy, which depends on irradiation directly of tumor or the surgical site by the high energy beam. Despite of applied treatment in some cases there is lack of response to the therapy or the cancer is invading local and distal tissues. One of the causes of cancer spreading is the variability of cancer cell population. There are cells, which respond and on the other hand some cells has developed the resistant mechanism towards applied treatment. The resistance can be transferred between non-responder cells to sensitive cells. For that occurrence are responsible double-membrane structures called exosomes. They play a crucial role in communication between various cells in local and distal localizations. Additionally, in healthy organisms exosomes are responsible for silencing immune reactions directed into healthy cells (prevents autoimmune diseases), secretion of toxic metabolites from intracellular environment, maternal-foetal tolerance. However, in case of tumors, it was observed increased secretion of exosomes equipped in various specialized proteins, which enable survival of cancer cells, silence immune response directed to cancer, decrease effectiveness of applied anticancer treatment. It was assessed, that exosomes can influence onto modification of local environment, where cancer occurs. The function of exosomes is related to the external factor which has impact onto cells. Studies over influence of ionizing radiation on glioblastoma cells indicated the increased secretion of exosomes, which were acting as a chemoattractant for non-irradiated cancer cells. What more, it was noticed, that exosomes induced by ionizing radiation were uptaken more favourable, than exosomes derived from non-irradiated cells. Those properties indicate the ability to modify local tumor environment. However, the influence of exosomes induced by ionizing radiation onto modification of cells, formation of invasive phenotype and breast cancer progression after irradiation is not well known process.

2. The purpose of the project:

The main goal of the project is to indicate the influence of exosomes induced by irradiation (exo - IR) onto invasiveness of cancer cells and their role in formation of invasive and more aggressive phenotype of cells in different molecular subtypes of breast cancer.

3. Materials and methods:

Exosomes will be isolated from cell culture medium by series of ultracentrifugations. The level of exosomal proteins will be evaluated by colorimetric assay BCA. To confirm the size and morphology of isolated exosomes scanning electron microscopy will be performed. The quality of isolated exosomes will be indicated by acetylcholinesterase assay. The established breast cancer cell lines will be incubated with exosomes induced by ionizing radiation to assess their role in modification of cancer cells. To evaluate changes induced by exosomes, the following assays will be performed: invasiveness of cancer cells (the overgrowth of artificial scratch in cell culture known as “wound healing assay”); invasion of extracellular matrix by cancer cells and zymography assay; evaluation of gene expression involved in migration, cellular stress, apoptosis, angiogenesis epithelial-mesenchymal transition. What more, the role of exo-IR in angiogenesis will be tested by vessel formation assays with usage of endothelial cells. Additionally the influence of exosomes induced by ionizing radiation onto proliferation, apoptosis will be performed with usage of flow cytometry kits.