Anna Nagel

The influence of tumour microenvironment on breast cancer cells in the context of changes within oestrogen receptor

Supervisor: dr hab. Anna Żaczek

Auxiliary supervisor: dr Aleksandra Markiewicz

Laboratory Of Translational Oncology

Institute of Medical Biotechnology and Experimental Oncology

Abstract

Breast cancer (BC) is one of the most common cancers among women worldwide. It can be divided into subtypes, where the most abundant are those which carry steroid hormone receptors (HR) for estrogen and/or progesterone. BC that lacks HR are one of the most aggressive with poor patients' outcome. Estrogen Receptor alpha is a 66kDa (ERα66) ligand-activated transcription factor involved in cell proliferation and is encoded by the *ESR1* gene. ER is also one of the most valuable prognostic and predictive markers in breast cancer assessed by immunohistochemical staining (IHC). Previously our team investigated its amplification in order to assess the prognostic value of *ESR1* gene dosage. Results indicated that ESR1 amplification may occur also in ER-negative patients and confers their poor prognosis. Because of this phenomenon we hypothesize, that high *ESR1* gene dosage may affect presence of other ER isoforms. ERα36 (Estrogen Receptor alpha 36) is an alternatively spliced variant of ERα, which lacks both transcriptional activation domains, but retains its DNA-binding domain. It is described as an unfavorable factor when expressed in cancer cells. Cancer associated fibroblasts (CAFs) are the most abundant cell type in the tumor microenvironment (TME), they may be involved in disease progression pushing cancer cells towards aggressive phenotype. The role of ERα36 in CAFs is totally unknown. The study aimed to discover the role of ERα36 in the context of different methods used to assess *ESR1* gene dosage and the action of ERα36 in the relation between tumor microenvironment and cancer cells.

For ERα36 and ERα66 gene expression analysis, 149 frozen primary tumor samples were used. The median age of the patients was 58 years (27–61 years). Informed consent was collected from all the participants who were included in the study. Twelve CAFs cultures were isolated from chemotherapy naïve BC patients and characterized for ERα36 expression, four representative CAFs lines were analyzed. Conditioned media from CAFs cultures were used to assess the influence of CAFs on BC cells using Matrigel 3D assay.

Methods used do assess the *ESR1* gene expression detects different aberrations in its locus. The expression of ERα36 and ERα66 isoforms was related to patients' survival, but their effect was opposite—high ERα66 levels conferred good prognosis, whereas high ERα36 conferred a poor prognosis. High expression of ERα36 isoform was an unfavorable prognostic factor in ER positive and ER negative BC patients. Breast cancer associated fibroblasts represent distinct subtypes, which might be characterized by ERα36 expression. ERα36-positive CAFs play an unfavorable role in ER negative BC cell line pushing them towards invasive phenotype.

Understanding the complicated dialogue between CAFs and breast cancer cells may open new ways for treatment and diagnosis.