Kinga Wilkus, MSc

The influence of tumor microenvironment on the activity and gene expression profile of organospecific endothelial cells

Dissertation for the Doctor of medical sciences and health sciences in medical sciences

Supervisor: Claudine Kieda, prof., PhD, DSc

Co-Supervisor: Klaudia Brodaczewska, PhD

Military Institute of Medicine-National Science Centre, Laboratory of Molecular Oncology and Innovative Therapies

The doctoral dissertation defense submitted to

Medical Sciences Discipline Board of the Medical University of Warsaw

Warsaw, 2023

Abstract

Introduction: Breast tumor is one of the most common cancer type among female population worldwide, still being the cause of thousands women's death. Thus, it is crucial to understand the mechanisms not only of tumor growth but also its progression and invasion. Angiogenesis, a process in which endothelial cells (ECs) are important players, is pathological in tumor effecting in blood vessels that do not function properly as in healthy tissue and this represents one of the hallmarks of cancer. The main goal of the project is to describe the influence of organo- and biological state- specificity of ECs, by their global and characteristic gene expression pattern. We demonstrate that ECs activity displays tissue-specific responses and that the cell model of tumor-derived ECs compared to healthy endothelium reflects the tumor microenvironment. Methodology: In this project, the study focused on the cells which are part of the tumor microenvironment and shape it during the disease i.e. the endothelial cells as they are responsible for the angiogenesis. The unique model of organospecific endothelial cells was used for the experiments. The cell lines were established from the endothelium of healthy tissue and primary tumor originating the same patient with breast cancer. These cells were immortalized in defined conditions which maintained their specific endothelial phenotype in terms of features and function according to the tissue origin. The cells were cultured *in vitro* in normoxia (21% pO2) and hypoxia (1% pO2). Functional assay, pseudo-tube formation, was used to evaluate the impact and significance of organospecificity on the activity of endothelial cells. Moreover, we used the cell viability assay to compare the proliferation rate of the cells in standard culture conditions according to their healthy or breast tumoral origin. Using flow cytometry and Western blotting, we characterised the ECs phenotype and the expression of selected molecules on the protein level. To determine how the microenvironment influenced important molecules as vascular endothelial growth factor A (VEGF-A) secretion by ECs, the level of VEGF-A was measured in medium by ELISA. Next generation sequencing (NGS) by the sequencing of the whole transcriptome, helped us to identify the key genes that are modulated by the tumor microenvironment. We identified a profile for a set of genes that indeed reflects endothelial tumor cells as distinct from normal tissues endothelial cells. Results: We review the current knowledge of ECs related to their organospecificity, plasticity and listed some selective angiogenesis models in pathologies. We presented characteristics and functions of ECs showing how endothelial progenitor cells or

endothelial precursor cells and mature ECs may be used in *in vitro* studies: in 3D models and co-culture with other cells to create a blood–brain barrier (BBB). Moreover, we sum up the role of endothelial cells in angiogenesis and pathologies. In the original paper, the global gene characteristics of the cell model was performed using whole transcriptome NGS. The most deregulated genes and biological processed between tumoral *vs* normal breast tissue-derived ECs, were identified. Pathological ECs were characterized by the increase of *Ephrin-B2* and *SNCAIP*, indicative of dedifferentiation and also lowered expression of CD31, EC marker. Therefore, other ECs specific proteins (ACE+, VWF+) and their differentiation markers (CD31+, CD 133+, CD105+, CD34), were assayed. We showed that their expression was downregulated in tumor-derived ECs. Moreover, pathological ECs had decreased levels of several other adhesion molecules (ICAM-1+, VCAM-1+), and barrier formation proteins as ZO-1+. By functional assays, such as pseudo-tube formation assay and permeability test, we confirmed the differences between both cell lines, what was also indicated by VEGF-A increased level released in response to low pO2. NGS data identified several genes involved in extracellular matrix (ECM) remodelling: collagens, laminin, fibronectin and integrin (ITGB6), as being deregulated in tumor-derived ECs. This further confirms pathological angiogenesis characteristics of HBCa.MEC evidenced in functional assays. Another process identified as altered in pathological ECs was endothelial to mesenchymal transition (EndoMT) correlated with the changes of ECM organization. Deregulated genes, included: *SPP1, ITGB6, COL4A4, ADAMTS2, LAMA1, GAS6, AGTR2, PECAM1, ELN, FBLN2, COL6A3, COL9A3*. ECM remodelling gene expression profile suggested that cancer ECs acquire migratory properties, what was later confirmed by functional assay- wound healing test (data not shown, under the preparation in the next publication).

Conclusion: The presently characterised unique model of breast tissue-derived ECs, representing the healthy and the tumoral tissue, demonstrates the necessity of proper cellular models to perform biologically relevant *in vitro* research. The endothelial cells differed significantly, both phenotypically and functionally, when originating from the tumor site as compared to the normal corresponding tissue. Not only the influence of the tumor microenvironment and the adaptive capacity of ECs were determined, but also their interactions with the stroma cells. This highlights the endothelial cells-to-microenvironment crosstalk which is crucial for tumor development: aside from disturbing angiogenesis, the tumor microenvironment rules deep phenotypic changes in pathological ECs by inducing EndoMT.