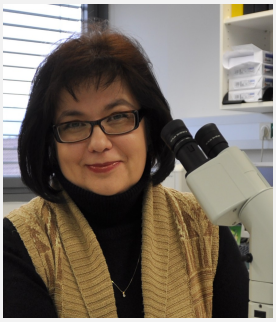
ImmunoTools *special* Award 2017



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Role of Cancer Stem Cells in Radiation Resistance

Radiation therapy (RT) is one of the main anti-cancer therapeutic approaches. Approximately 80-90% cancer patients receive RT either in curative or palliative settings during the disease history. Despite the fact that RT is highly effective and can kill the majority of cancer cells within the malignant tumor, the problem of radiation resistance is still not solved. Currently, the majority of cancer researchers support a cancer stem cell (CSC) theory as a background not only for carcinogenesis but also for intrinsic (primary) and acquired (secondary) treatment resistance. Thus, it is suggested that local and distant relapses occur from cells survived after therapy. Hence, it is logical to assume that evaluation of the molecular properties of CSCs can help to predict and/or combat development of the tumor recurrences. In order to elucidate the CSC molecular patterns, CSCs should be harvested from the non-CSCs and undergone further investigations using a variety of omics-methods. Unfortunately, CSC purification either from cancer cell lines or from the human tumor samples reveal some limitations due to the absence of the definitive CSC markers. However, CSC carrying currently existing markers (CD44, CD24, ALDHA1, CD133, etc.) could be isolated using flow cytometry or magnetic cell separation for further investigations of their molecular and physiological properties. For CSC isolation we will need the labeled antibodies for CD24, CD44 molecules.

Recently, our research group has developed two in vitro models of carcinoma cells of different origins (breast cancer, head and neck squamous cell carcinoma, prostate cancer) with enhanced radiation resistance and increased metastatic properties. Both cellular models demonstrated enrichment for CSCs resulting in the more efficient experimental work with CSCs characterized by radiation resistance and metastatic capacities. Additionally, we have found that radioresistant and metastatic CSCs are characterized by the increased number of extracellular microvesicles (including exosomes) containing CSC-specific proteins. It is suggested that exosomes released by CSCs can change molecular and physiological phenotypes of radiation-sensitive carcinoma cells resulting in the enhancement of tumor radiation resistance. In order to prove our hypothesis, we plan to isolate CSC-specific exosomes and to check whether they can affect radiation response in the treatment-sensitive carcinoma cells. To confirm the quality of the exosome isolation, expression of the exosome-specific biomarkers should be evaluated (CD63 and CD81). Isolated exosomes will also be

analysed for their protein content using proteomic approach. The proteins of interest identified in CSC-specific exosomes can help to find additional CSC markers helping to predict unfavorable treatment outcome in patients with radioresistant tumors. In order to prove the physiological role of the identified proteins in the development of radiation resistance, the recombinant proteins will be used in a number of experiments (cell viability assay, clonogenic assay, sphere-forming assay, evaluation of metabolic activities of cells, influence on the intratumoral angiogenesis, etc.).

For these experiments not only the recombinant proteins and cytokines will be needed, but also ELISA kits and labeled antibodies for flow cytometry analyses will be required to determine how the proteins of interest change the behavior of CSCs and non-CSCs. Since tumor exosomes are released into the biological fluids, we plan to investigate exosomes isolated prom plasma (prostate, breast and HN cancer patients) and saliva (HN cancer patients) and to determine which proteins belonging to the CSC-associated exosomes could be used as predictive biomarkers.

This is a comprehensive translational study helping to develop the putative biomarkers predicting higher intratumoral CSC content and unfavorable treatment outcome in cancer patients.