ImmunoTools *FlowISiAM* Award 2026

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Biomarkers of progression and chemoradiotherapy side effects toward personalized treatment of cervical cancer

Background

Prediction of radiosensitivity in cervical cancer patients with the radiation induced lymphocyte apoptosis (RILA) method

Cervical cancer remains one of the most common cancer with bad prognosis affecting women worldwide, primarily developing as a result of HPV infection. The virus causes normal epithelial cells to undergo cancerous transformation. Unfortunately, many women still do not receive the HPV vaccine, leaving them vulnerable to infection and its consequences[1]. In the treatment of early-stage cervical cancer radiotherapy is a crucial part of the treatment, though it can cause severe side effects that significantly diminish patients' quality of life[2].

Our research group focuses on radiobiological studies, aiming to develop a method that can easily and quickly determine whether a patient is radiosensitive before starting treatment. Since radiotherapy is common in cervical cancer patients and they often experience severe side effects[3], we choose this patient group to establish the RILA method in our laboratory. The RILA (Radiation Induced Lymphocyte Apoptosis) method appears to be a promising and simple method for detecting radiation sensitivity. This method is one of the few used for determining individual radiosensitivity, which is based on multicenter studies performed on thousands of patients[4]. The measurements involve examining the frequency of $CD8^+$ and $CD4^+$ lymphocytes in peripheral blood that undergo radiation induced apoptosis. RILA is a negative predictor, patients with low RILA values, indicating a low number of apoptotic cells, are more likely to develop late side effects than patients with high RILA values, who have a higher number of apoptotic lymphocytes[5].

The biomarker potential of TKTL1 and Apo10 in cervical cancer

TKTL1 overexpression in cervical cancer cells was demonstrated in previous studies using cell cultures[6,7] and tissue samples[8]. Elevated transketolase activity resulted in increased proliferation, and Zhu et al demonstrated that by silencing TKTL1, the potential for migration and invasion decreased and apoptotic activity increased. They also confirmed TKTL1 involvement in tumor growth in a mouse model of cervical cancer[7]. A semiquantitative immunohistochemical staining study demonstrated that TKTL1 staining intensity shows an increasing tendency with histological grade in cervical tissue samples[8] and showed promising diagnostic performance in precancerous cervical lesions[9,10]. DNaseX (Apo10) is also increased in tumor cells[11–13], including cervical cancer and cervical intraepithelial neoplasia[14]. DNaseX is involved in apoptosis, although in cancer cells it is either inactive or inhibited[15].

To the best of our knowledge, there is only one study which evaluate Apo10, TKTL1, and APT (combined Apo10 and TKTL1 score) in screening early-stage cervical cancer using the epitope detection in macrophages (EDIM) method. They concluded that Apo10, TKTL1, and APT are promising diagnostic markers, although their study was limited to patients with early-stage disease[16] TKTL1 was shown to have potential as a prognostic marker of progression in different types of cancer[17–19], but as of yet, TKTL1 and Apo10 together were only tested once as prognostic markers in oral squamous cell carcinoma (OSCC), where TKTL1 and Apo10 positivity correlated with poor overall survival, especially when the patient was positive for both[20]. This study, however was not using the EDIM method to test TKTL1 and Apo10 as prognostic factors, and was conducted on tumor samples, not on blood macrophages. However, they did compare EDIM scores of OSCC to healthy controls, and found increase of EDIM scores in OSCC cancer patients. These results raise the possibility, that detection of $TKTL1^+$ and $Apo10^+$ macrophages form patient blood samples may lead to the discovery of promising, non-invasive prognostic markers.

Aims

We would like to test our cervical cancer patient cohort blood samples using the *FlowISiAM* AT-test to measure TKTL1 and Apo10 levels, and determine whether they can be used as prognostic markers of cervical cancer. We plan to correlate TKTL1 and Apo10 results with RILA data to determine if they can be useful complements as predictive markers of radiation sensitivity besides $CD4^+$ and $CD8^+$ lymphocytes. We would like to recruit 50 more cervical cancer patients and 50 healthy individuals and apply the RILA and *FlowISiAM* AT tests on them as well.

Experimental design

For our study we recruit patients scheduled to undergo definitive radiochemotherapy with/without immunotherapy. We collect blood samples before radiotherapy, at 3 months, 1 year, and 2 years after treatment completion. RILA is measured only in the sample collected before radiotherapy. At every time point, PBMC and plasma samples are collected, and we assess side effects using EMBRACE II, EORTC-QoL-C30, and EORTC-Cx24 questionnaires. In addition to prediction of the side effects, we aim to find patients with great progression risk. We would like to perform Apo10, TKTL-1 measurements on these PBMC samples for *FlowISiAM* AT-test and *AMANDUS*. Our patient cohort includes 187 patients, and we would like to recruit 50 more. These patients are closely followed, and comprehensive clinical data are available for all participants. This enables us to accurately identify responders and non-responders to therapy, as well as to determine the progression free and overall survival. We want to investigate how the levels of Apo10 and TKTL1 change during therapy and to evaluate their potential as prognostic biomarkers for cervical cancer.

We also plan to measure Apo10 and TKTL1 in 50 healthy volunteers to compare the results with those of the patients. Apo10 is involved in apoptosis in normal cells [21], and in

HEK293 cells, and TKTL1 was shown to have a protective role against apoptosis [22]. As both *FlowISiAM* AT-test and RILA method applies apoptosis measurements, but in different leukocyte types (RILA is measured in lymphocytes and *FlowISiAM* AT-test measured in monocytes/macrophages), it would be of interest to correlate Apo10 and TKTL1 levels with the patients' RILA values, since the RILA assay quantifies normal T lymphocytes that have undergone apoptosis.

As a result, with RILA and extended *FlowISiAM* AT-test we will be able to combine a prognostic test of outcome and a predictive test of side effects for cervical cancer patients, which could facilitate personalized patient management.

Collaboration plan

For this research we would like to kindly ask SMEs ImmunoTools and partner INVIGATE to provide antibodies for the AT-test and some other reagents for flow cytometric research, and to support the establishment of *FlowISiAM* in our laboratory.

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