ImmunoTools *special* Award 2014

Xiaoyan Wang, PhD student

Supervisor: Prof. Dr. Christiane J. Bruns

Klinik für Allgemein-, Viszeral- und Gefäßchirurgie
Universitätsklinik Magdeburg A.ö.R.
Leipziger Straße 4, 39120 Magdeburg

Preclinical research of Immune targeting cancer stem cells in Esophagus Cancer

Esophageal cancer including esophageal squamous carcinoma (ESCC) and esophageal adenocarcinoma (EA) is an extremely deadly malignancy with 5-year survival rates of 5% to 30%. Currently, relatively little is known about esophageal cancer initiation, growth, maintenance and progression.

Recent studies have suggested that human solid tumors may contain subpopulations of cancer stem cells (CSC) with the capacity for self-renewal and the potential to initiate and maintain tumor growth. Several techniques have been used to enrich and identify CSC, including growth in serum-free defined media to induce sphere formation, and isolation of a stem-like cell using exclusion of the fluorescent dye Hoechst 33342, the side population (SP). The side population (SP) cells have been exploited as a marker to identify and purify stem cells from a variety of tissues, and as a model for cancer stem cell functional study. Cancer stem cells have been identified in several solid tumors, but stem cells in normal human esophagus or adenocarcinoma have not been reported. Currently, SP cells are usually used to study the esophagus CSCs and its resistance to radiotherapy and chemotherapy.

The immune system plays an important role in the regulation and outcome of cancer. And immunotherapy has become a promising therapeutic strategy for a variety of cancers over the past decade. My interests are to investigate the function of immune system in esophageal cancer initiation, growth and metastases. We will employ a combination of approaches including quantitative real-time polymerase chain reaction (qRT-PCR), immune blotting, immunohistochemistry (IHC), flow cytometry as well as immunofluorescence to address this question. Understanding this process will greatly improve our ability to develop novel immunotherapeutic strategies for esophageal cancer.

Two parts of my subject will be completed by utilizing the reagents from ImmunoTools.

Part one: to investigate the role of immune cells in esophageal squamous cell carcinoma (ESCC) development.

We will use 4-nitroquinoline-1-oxide (4-NQO) to induce experimental esophageal squamous cell carcinoma (ESCC) in C57B1⁄6 female mice. During the ESCC development (2, 4, 8, 12 and 16 weeks after 4-NQO treatment), we collect the immune cells from lymph node (superficial cervical nodes, axillary nodes, renal nodes, mesenteric nodes and inguinal nodes) and spleen. The phenotypes of immune cells will be analysed through magnetic cell isolation and flow cytometry (FITC-conjugated Annexin V, FITC-conjugated anti-mouse CD3e, CD11b, CD44, CD45R, isotype control IgG2b, PE-conjugated anti-mouse CD8a,NK-cells, CD45, APC--conjugated anti-mouse CD4, CD19, Gr-1). Based on this experiment's data, we will try to discuss the role of immune cell in ESCC.

Part two: to explore potential immunotherapy strategy through human immune cell mediated side population (SP) cell killing experiments.

In our previous study we have report that stem cell like side populations in esophageal cancer is a source of chemotherapy resistance and metastases (Stem Cells Dev. 2013, PMID:24021093).Due to the existence of the SP or cancer stem like cell, the prognosis of esophageal cancer is quit poor. To search for a novel immunotherapy targeting SP cells may be a good way.

Firstly we isolate SP cells and non-SP cells from esophageal adenocarcinoma cell lines OE19, OE21, OE33 through flow cytometry based on Hoechst 33342 staining. At the same time, we will detect the expression of some stem cell biomarker including CD105 and CD44 through flow cytometry using FITC-conjugated anti-human CD105 and PE-conjugated anti-human CD44 (from ImmunoTools). Secondly, we will use recombinant human cytokine (rh IL-4, and rh GM-CSF) to induce DC differentiation from monocyte, and utilize magnetic cell isolation and flow cytometry (FITC-conjugated anti-human CD4, CD45RA, PE-conjugated anti-human IL-6, PerCP-conjugated anti-human CD3, APC-conjugated anti-human CD8, Control-IgG2b) to isolate T cells from human PBMCs. Then we use the cell lysates of SP cells or non-SP cells to treat DC cell respectively, which will be submit to T cell mediated cancer cell killing experiments together with isolated T cells and SP cells.

Besides, the CXCL12/CXCR4 axis are now considered to play an important role in the metastasis of various malignancies, therefore we also want to investigate the pathologic role of CXCL12/CXCR 4 axis in EC and the potential therapeutic

strategy of targeting this axis for the treatment of EC by using rh SDF-1α/ CXCL12a, rh SDF-1β/CXCL12b, IL-29 and rh RANTES/CCL5.

ImmunoTools *special* AWARD for **Xiaoyan Wang** includes 25 reagents

FITC - conjugated anti-human CD4, CD45RA, CD105, Annexin V

PE - conjugated anti-human CD44, IL-6,

PerCP - conjugated anti-human CD3,

APC -conjugated anti-human CD8, Control-IgG2b,

recombinant human cytokines rh IL-4, rh GM-CSF, rh SDF-1α/ CXCL12a, rh SDF-1β/CXCL12b, IL-29, rh RANTES/CCL5,

FITC - conjugated anti-mouse CD3e, CD11b, CD44, CD45R, isotype control IgG2b,

PE - conjugated anti-human CD8a, NK-cells, CD45,

APC -conjugated anti-human CD4, CD19, Gr-1,

DETAILS