GESINAS - ImmunoTools Award 2015

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Cell Immune Interaction with Cancer-Initiating-Cells and Tumor Microenvironment in Gastrointestinal Tumours

Research background

As a surgical oncology research group we have been focussed on characterization of cancer stem cells (CSC) isolated from a series of upper GI cancer cell lines (including pancreatic cancer, liver cancer, and oesophageal cancer). According to their resistance to chemo- and/or radiotherapy and their higher potential to initiate tumors as compared to non-CSC populations, CSCs were considered as key factor for tumor relapse. In addition, the tumor microenvironment (TME) has gained increasing attention because it may work as a double-edged sword to control tumor progression.

Scientific objective

We hypothesized that the immunological profile of GI-CSCs are differently expressed as compared to non-CSCs and might be dynamically changing in different stages of the disease or following conventional therapies, which may be in part responsible for failure of immune surveillance of cancer. Therefore, we want to identify the immunological profile of GI-CSCs as well as their chemotherapy resistant variants and their interaction with TME generally constituting aside of stromal components of angiogenic vascular cells (AVCs), infiltrating immune cells (IICs), cancer-associated fibroblastic cells (CAFs), regulatory T cells and TAMs (tumor-associated macrophages).

Work plan

1. Isolation of GI-CSCs and non-CSCs from different GI-cancer cell lines and establishment of chemotherapy-resistant variants

PDAC, HCC and EAC cell lines and their respective selected chemotherapy resistant sublines will be examined and sorted for CSCs and non-CSCs by flow cytometry either by surface marker antibody based staining (CD133, CD44, ESA, CD24,

CXCR4, CD90 and ABCG2) or with functional assays such as Hoechst 33342 staining for side population or by ALDEFLUOR™ assay for ALDH enzymatic activity staining according to our routine protocol.
In addition, one mouse PDAC cell line (Panc02) and its gemcitabine resistant variant were also applied in this study for checking mouse surface markers as well as ALDH-1 and SP proportions (for further immune modulation research).

2. Detection of immuobiological molecules of CSC, non-CSC and chemotherapy resistant variants

The expression of MHC-I, MHC-II, NKG2DLs (MICA/B, ULBP-1, ULBP-3, ULBP-3, ULBP-4), B7-H1(PD-L1) will be detected in both CSC and non-CSC different culture/differentiation stages by cytometric analysis.
Alteration of antigen processing and presentation is a common event in cancer progression. Therefore, we plan to check the APM expression by intracellular cytofluorimetric analysis of MHC class I molecules and the heavy chains, immunoproteasome (LMP2, LMP7, LMP10).

3. Detection of cytokines or growth factors from isolated CSCs, non-CSCs and their chemotherapy resistant variants

These cells will be cultured in serum free medium for 24h and 48h up to an appropriate confluency to further collect the supernatants. IL-6, IL-8, IL-10, TGF- β and VEGF will be detected by human ELISA kit.

4. Immune response of CSCs and non-CSCs of Panc02 in C57/BL6 mice.

Organs (spleen, pancreas with or without tumors, macroscopic metastatic lesion) and blood will be harvested from healthy control mice, mice following orthotopic injection of Panc02 cells, Panc02-CSC, Panc02-non-CSC as well as their chemotherapy resistant variants for the following analysis:
T cells: CD3, CD4, CD8
Treg cells: CD4 CD25 Foxp3
MSC cells are planned to isolated from both mouse bone marrow and adipos tissues: CD11b, CD11c, CD29, CD34, CD45R, CD44, Sca-1 and CD117
Additional immune markers are planed to be detected in tumor and tumor/ pancreas margin tissue by immunostaining of CCL5, SDF-1, CXCR4, IL-12, IL-23, CCL18, CCL22, FoxP3 in frozen sections (tumor associated macrophages and regulatory T cells infiltrated in primary tumors)

<u>Significance and outlook</u>
We will compare a panel of immunobiological markers including MHC, tumor antigen and the secreted factors from CSC and non-CSC subpopulations in a series of GI cancer cell lines. It will reflect the immunogenicity of CSCs as well as the immune response capacity, which may provide some implications for immunotherapy

overcoming CSC associated chemotherapy resistance in terms of a cancer microenvironment modulation strategy.

If applicable, we will also detect the immunobiological profiles (significantly dysregulated markers optimized from above cell lines or animal model) of patient-derived tumor cells from tissue or circulation (Known as CTC) with or without adjuvant chemo therapy as well as the infiltrated lymphocytes in the future process.

GESINAS-Award application:

The educational assistance and support on developing Children's interest at STEM (Science, Technology, Engineering, and Mathematics) and art fields is my big social focus. I have been actively involved in the teaching or volunteer work since 2004 for different museums (Children discovery museum, Science and Tech Museum), SCI (Service Civil International), primary schools (education programs for Global Business lectures, biomedical science and laboratory tour and traditional Chinese culture) as well as other NGOs' services. On the one side, these activities inspired me a lot for keeping passion and developing personal capacities to help the young generation in particular for their potential interests in a scientific career. On the other side, I realized that it is an emergent task to encourage more clinicians and scientists to bring their profession into public education.

References:

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2. Di Tomaso T1, Mazzoleni S, Wang E, Sovena G, Clavenna D, Franzin A, Mortini P, Ferrone S, Doglioni C, Marincola FM, Galli R, Parmiani G, Maccalli C. Immunobiological characterization of cancer stem cells isolated from glioblastoma patients.Clin Cancer Res. 2010 Feb 1;16(3):800-13

3. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 2009 Nov;86(5):1065-73

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5. Michael Quante, Julia Varga, Timothy C. Wang and Florian R.Greten. The Gastrointestinal Tumor Microenvironment. Gastroenterology. 2013 Jul; 145(1): 63–78.

GESINAS ImmunoTools AWARD for **Yue Zhao** includes 42 reagents

FITC - conjugated anti-human CD34, CD44, IL-6, Annexin V

PE - conjugated anti-human CD38

Multicolour combinations anti-human:

CD4 FITC / CD3 PE / CD8 PerCP

recombinant human cytokines: rh BMP-7, rh EGF, rh FGF-b, rh GM-CSF,

rh IFN- gamma, rh IL-6, rh IL-7, rh IL-17A, rh IL-17F, rh SDF-1a

human ELISA-set for 96 wells,human IL-6, IL-8, IL-10 (each 3 reagents)

FITC - conjugated anti-mouse CD3, CD8a, CD11b, CD29, CD117, isotype control IgG2b

PE - conjugated anti-mouse CD4, CD34, CD45R, NK cells, isotype control IgG2b

APC - conjugated anti-mouse CD25, CD44, isotype control IgG2b

mouse ELISA-set for 96 wells, mouse TNF-a (each 3 reagents)

DETAILS more AWARDS