Novel techniques in gynecological oncological diagnostics and experimental design.

PhD thesis

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Budapest

2023.

Introduction

Immunological interaction between Natural Killer cells and Trophoblastic cells: Decidual natural killer cells (dNK) have been the focus of many studies because of their unique roles in both the anti-tumor immune response and healthy placental formation. Revealing the immunological mechanisms by which they interact with their target cells may lead to a better understanding of immune evasion of certain tumor cells, including abnormal cells of the different forms of gestational trophoblast disease and miscarriages of immunologic origin. Efforts to perform functional immunological studies on dNK cells have been limited by difficulty obtaining sufficent quantities of cells and sustaining the dNK phenotype. In gestational trophoblastic neoplasia (GTN) trophoblastic cells show an uncontrolled prolypheration and propagation into the maternal tissue.

New role of CA 125 in diagnostics: By immunizing mice with an epithelial ovarian cancer cell line and harvesting spleen cells, a monoclonal antibody was identified that reacted with sera from a majority of women with epithelial ovarian cancer. In papers describing the discovery, the antibody identified was called OC125 and the antigen it detected, CA125. CA125 was known to be part of a much larger glycoprotein, which was

eventually identified as a member of the mucin family of proteins (MUC16). Besides serial measurement of CA125 to predict recurrence, initial levels of CA125, combined with other markers, are approved for the differential diagnosis of pelvic masses. Observations suggest that MUC16 has a much more active role in promoting disease progression. It is known that CA125 can bind to mesothelin to promote adherence of ovarian cancer cells to mesothelium and spread intra-abdominal disease. Secondly, CA125 can bind to siglec-9 receptors on peripheral blood mononuclear cells (PBMC) including NK cells and blunt their tumoricidal ability.

Objectives

My goal was to create a novel protocol for dNK cell isolation from placental and complete hydatidiform molar (CHM) tissue samples. These dNK cells should present good viability and be sustainable in cell culture for further experiments. It was also my objective to create an ex-vivo experimental design to observe the immunological interaction between dNK cells and different trophoblastic cells. Choriocarcinoma cells may cause alteration of the dNK cell function or phenotype compared to non neoplastic trophoblastic cells.

Leukocytes are able to move in and out of the circulatroy system and enter tumorous tissue. They bind CA125 on their surface both in the serum and the tumor micro-environment. These leukocytes can be isolated from the peripheral blood and among their subtype, the number of CA125 bound to their cellular surface can be assesed. With a novel microscope system (developed by PNP Reserach, Thorlabs, University of Wisconsin et al.) my goal was to perform a longitudinal measurment of the CA125 binding capacity of various leukocyte subtypes.

Methods

For the dNK cell isolation protocol, end-term, fresh placentas were selected (n=10) of uneventful pregnancies. CHM samples were also selected (n=2). The selected tissue samples were processed in order to gain a high concentartional cell suspension. The cells were stained by CD 3, CD 14, CD 15, CD 56, CD 16 and DAPI staining. CD 56 bright, CD 16- cell were isolated as dNKs. Functional and phenotypical analysis were performed.

The isolated dNK cells were exposed to benign (HTR-8) and malignant (Jeg-3) trophoblastic cells for 1 and 5 days. After the exposure time the cells were lifted and dNK cells were sorted back. Phenotypical anaylsis of the exposed dNK cells were made and compared to controls of the same pool, without exposure. Standard cytotoxicity assesment was also done comapring the citotoxicity of the exposed dNK cells to controls.

For the longitudinal assesment of CA125 bound to different leukocytes a patient diagnosed with a high-grade epithelian ovarian cancer (stage IIIC) was selected. Cryopreserved peripheral Blood Mononuclear Cell (PBMC) were used. The PBMCs were stained with fluorophore conjugated anti

leukocyte marker antibodies (CD 45, CD 19, CD 56, CD 14, CD 3) followed by incubation with anti CA125 conjugated gold plasmonic nano particles (PNP). Each leukocyte subset's mean PNP binding capacity was measured and followed during a 21 month- period.

Results

dNK cells from the fetal- maternal interface and CHM tissue were acquired. In the case of end-term placenta, we acquired an average of 160,000 uNK cells/ minute and an average of 140 000 uNK cells per gram of starting placental material. Regarding the CHM tissue the purity of the cell suspension permitted acquisition of an average of 80 000 uNK cells/ minute. and an average of 70 000 uNK cells per gram of starting CHM tissue.

After co-culturing the dNKs with HTR-8 and Jeg-3, we observed no changes in the phenotype of the dNK cells after 1 day of exposure but 5 days of exposure changed the phenotype of the dNK population exposed to Jeg-3 to be similar to the peripheral NK cell phenotype. We also found decreased cytotoxicity of dNK cells co-cultured with Jeg-3 cells compared to the dNK population exposed to HTR-8 and the controls.

Regarding the bound CA125 a large number of fluctuations are seen in the PNP binding over time to cell type. Potential linkage with clinical events was observed. Over a 21-month period, monocytes had the highest level of MUC16-binding which was positively correlated with serum CA125 and inversely

correlated with circulating monocyte and lymphocyte counts. Fluctuations of PNP-binding to NK cells were associated temporally with types of chemotherapy and surgical events. Levels of MUC16 bound to NK cells were positively correlated with levels of MUC16 bound to T and NK-T cells and inversely correlated with circulating platelets.

Conclusions

This new protocol is a reliable technique for obtaining high quality and viable leukocyte populations from the placenta. Our data suggest that the protocol is effective in obtaining high number of viable NK cells from CHM tissue as well.

While dNK cells undergo various changes in their function after being exposed to the invading eVTs, alteration of their cytotoxicity is only one aspect of it. The alteration of the NK phenotype, combined with decreased cytotoxicity in the group that was exposed to Jeg-3 supports our original hypothesis that the chroriocarcinoma tissue creates a more suppressive micro environment for NK cytotoxicity. Unraveling the nature and exact mechanism of this effect will be an important area for future study.

Assessment of MUC16-binding among cryopreserved PBMC cell types can be accomplished using darkfield and fluorescence microscopy. Correlations observed between level of binding by cell type with serum CA125, complete blood count data, and treatment details suggest that the new techniques may offer novel insights into epithelial ovarian cancer clinical course.

Bibliography of the candidate's publications

Related to my thesis:

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