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Adoptive T Cell Therapy for Metastatic Colorectal Cancer

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Adoptive T Cell Therapy For Metastatic Colorectal Cancer

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Date: August 8, 2013
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Instructor: Bo Xiang, PhD Scholar
Undergraduate Institution: Lincoln University, PA
Overview

- Colorectal Cancer
- Antigen: Guanylyl Cyclase C (GCC)
- T Cells and T Cell Receptors
- Adoptive T Cell Transfer
- Viral Vector Vaccine
- T cell Hybridoma Technology
- Results
- Conclusion
- Future Directions
Colorectal cancer commonly called colon cancer or bowel cancer is the 4th most frequent cause of cancer in the United States and it is the 2nd leading cause of cancer-related deaths in the United States.
Stages of Colorectal Cancer

Stage 0 -- Cancer is only in the innermost lining of the colon or rectum.
Stage I -- Cancer has not spread beyond the inner wall of the colon or rectum.
Stage II -- Cancer has spread into the muscle layer of the colon or rectum.
Stage III -- Cancer has spread to one or more lymph nodes in the area.
Stage IV -- Cancer has spread to other parts of the body, such as the liver, lung, or bones.
Colorectal Cancer

<table>
<thead>
<tr>
<th>stage</th>
<th>Chance of cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80-95%</td>
</tr>
<tr>
<td>II</td>
<td>55-80%</td>
</tr>
<tr>
<td>III</td>
<td>40%</td>
</tr>
<tr>
<td>IV</td>
<td>10%</td>
</tr>
</tbody>
</table>
Local vs Metastatic Colon Cancer

Colon Cancer

Local:
Those that remain in the intestinal lining and can be easily removed using surgery

Metastatic:
The cancer has spread through the blood and lymph nodes to other parts of the body. Major treatment is chemotherapy.

OUR APPROACH

Using T-Cell Based Immunotherapy to Target Metastatic Colon Cancer
Guanylyl cyclase C is a transmembrane receptor with high expression in intestinal epithelial cells.

Guanylyl cyclase C, a member of guanylyl cyclase family of receptors, is the receptor for two endogenous ligands guanylin and uroguanylin and for the exogenous ligand heat-stable enterotoxin, ST. Its main function is fluid hemostasis and electrolyte homostasis. As a mucosa specific protein, which is so highly restricted in its expression, GCC can be utilized to detect metastases in non-intestinal tissues.
T cells are activated by binding of the T cell receptor (TCR) to a peptide-major histocompatibility complex (MHC) complex (pMHC) expressed on the surface of antigen presenting cell.

When T cell receptor is engaged:
1. Signals are transmitted
2. T cells becomes activated
3. T cell expresses activation markers
4. T cell produces Immune factors
5. T cell proliferates
6. T cell kills target cells
Adoptive T Cell Transfer

• Process that involves:
  1) Removing lymphocytes (in this case the T cells) from the patient
  2) Tranducing the TCR of the T cells
  3) Grow them to large numbers
  4) Return Cells to the Patient
Project:
Cloning GCC specific TCR
Viral Vector Vaccines

1. Adenovirus
   - Cancer antigen

2. DC

3. T cells
   - CD80
   - CD40

Cancer antigen: GCC-Ad

proliferation

effectector
T-hybridoma Technology Diagram

- T Cells from Spleen
- Fusion Partner
- Antigen

1. Fuse in polyethylene glycol
2. Select and grow hybrid cells using HAT media
3. Test for antigen specific positive T cells
4. Propagate clones
T-Hybridoma Technology

Explanation

- The picture is showing a mouse being immunized with a viral vector vaccine. Once the mouse has mounted an immune response, the spleen is harvested and the splenocytes (site of T cells) are isolated.

- These isolated T cells are then fused with thymoma cells that are being grown in a culture by using polyethylene glycol (PEG). The fusion product is called T-hybridoma.

- HAT is then added to kill non-fused cells and then selection of growing T-hybridoma is done at a later date.

- Test of antigen specific T cells are then done using MUG assay.
Procedure

Mice were given the GCC-Ad5 vaccine

At D14, Spleen from immunized mice harvested and cells collected.

Immortal cancer (BWZ/CD8) cells grown in medium

Fusion of both cells in 1:1 ratio using PEG

Some splenocytes were collected in 3 flasks for 1 wk, 2wk and 3wk stimulation with GCC-peptide and RhIL-2.

HAT added at D1 and D7 to kill non-fused cells

At D12, growing t-hybridomas transferred to 24-wells.

Some splenocytes were collected in 3 flasks for 1 wk, 2wk and 3wk stimulation with GCC-peptide and RhIL-2.

At D16, MUG assay done to select GCC T-hybridoma using DMSO as a control
Results

- Growing T-hybridoma were obtained but none that were GCC specific.
Summary of project

- T hybridoma technology has proven to be an effective way to identify antigen specific TCRs.

- However in order to increase fusion efficiency for tests, critical measures have to be revised: During the fusion reaction, maintaining the temperature at 37°C is very important for the membrane events that occur during and immediately after the PEG mediated fusion event. Because of the small volume, the cells can rapidly come down to room temperature.

- In addition, the thymoma cells could also be tested to see if they are still potent for the method.
Future Directions

- Clone out the T-cell receptor of GCC specific CD8 T Cells and test their affinity.
- Give mice colon cancer cell lines-CT26 modified to express GCC and then treat them with GCC-specific TCR transduced CD8 T Cells.
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Are There Any Questions?