Drug delivery system with anaerobic bacteria for cancer therapy

2019/10/17
M2 Takahashi Kazuki
Contents

1. Well-known cancer therapies

2. Anaerobic condition in solid cancer tumors

3. Bacterial immunotherapy for cancer

4. Applications of anaerobic bacteria as a novel drug delivery system

5. Summary
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5. Summary
Orthodox method to treat cancers

- Surgery is performed in the limited areas in the body.
- Some solid tumors are resistant to radio- & chemotherapy.

Photo by JAFAR AHMED on Unsplash
Downleft: scienceblog.cancerresearchuk
**Immunotherapy**

- **Dendritic cell therapy**
  - Cancer vaccine

- **Cytokine therapy**
  - Interferon
  - Interleukin (IL-2)

- **Antibody therapy**
  - Antibody-dependent cellular cytotoxicity

- **Immune checkpoints**

Drug delivery systems to solid tumors

• Nanoparticle (NP)

Matsumura Y, et al., Cancer Sci., 2009, 100, 572

• Antibody-Drug Conjugate (ADC)


• These drug delivery systems are not effective for some solid tumors.

• A novel DDS is required to be invented to cope with any solid tumors.
Summary of Well-known cancer therapies

• Many types of cancer therapy were developed and are being developed now.

• Some solid tumors are resistant to various therapies.

• A new type of DDS is required to be established to cope with the solid tumors.
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1. Well-known cancer therapies
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Solid tumor characteristics

• Hypoxia: anaerobic environment

• Oxygen pressure
  • Normal tissues: 3-5% and 20-100 mmHg
  • Solid cancer tissues: <1% and 0-20 mmHg

• Angiogenesis: supplying several nutrients and oxygen and metastasis.

• How is such a low oxygen condition created despite angiogenesis in evident enhancement of malignant tumors?
Solid tumor characteristics

• Hypoxia: anaerobic environment

• Oxygen pressure
  • Normal tissues: 3-5% and 20-100 mmHg
  • Solid cancer tissues: <1% and 0-20 mmHg

• Angiogenesis: supplying several nutrients and oxygen and metastasis.

• The angiogenesis is outpaced by the tumor growth.
• The blood vessels formed by angiogenesis don’t have ability to supply enough O$_2$. 
Hypoxia

- HIF-1 (Hypoxia-inducible factors)
  - Stabilized by hypoxic conditions
  - Upregulates glycolysis enzymes and VEGF

glycolysis enzymes $\rightarrow$ ATP synthesis
vascular endothelial growth factor (VEGF) $\rightarrow$ angiogenesis.

the 2019 Nobel Prize in Physiology or Medicine
William G. Kaelin Jr., Sir Peter J. Ratcliffe
and Gregg L. Semenza
for their discoveries of how cells sense and adapt
to oxygen availability
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Bacterial therapy for cancer

• The original observation of tumor spontaneous recovery and regression of certain cancer patients from concurrent bacterial infections was made over 300 years ago.

• Numerous case reports, review papers and book chapters on the subject have been published, including about 1000 cases by the year 1987 and mean 10 reports per year by 2003.

M.Q Wei et al., Cancer Letters, 2008, 259, 16
Bacterial therapy for cancer

- Concept

Anaerobic bacteria

i.v. injection

A solid tumor cell in anaerobic condition → The bacteria live in the solid tumors only → The growth of bacteria → Oncolytic effect!!
Phase 1 study of *Salmonella* VNP20009

- 25 patients received an intravenous dose of VNP20009.
- The maximum tolerated dose (3x10^8 cfu/m^2)
- Shrinking of the tumor was not observed in any case.
- The existence of bacteria in the tumor was detected in only three patients.
- In four patients, bacteriosis was observed but the bacteria were not detected in the tumors.


This clinical test was failed.
Anaerobic bacteria grows in tumors only.

- *Clostridium tetani* produces toxins.

- The injection of *Cl. tetani* spores resulted in tetanic death in the tumor-bearing host in approximately 48 hours, regardless of the tumor size, the tumor type, or the spore dose.

Anaerobic bacteria grows in tumors only.

- *Clostridium tetani* grew a lot in the tumors only.

- Numbers of the bacteria in other organs were not different between in tumor-bearing mice and non-tumor-bearing mice.
Bifidobacterium

- Bifidobacterium has long been prescribed for infant patients in Japan.
- It was considered as safe bacteria injected intravenously.
Bifidobacterium grows only in tumor tissues

Fig. 1. Specific distribution of Bifidobacterium bifidum (LacB) in tumor tissues following a single i.v. injection of $5 \times 10^6$ viable bacilli into Ehlich solid tumor-bearing mice. Each point represents the mean of the number of bacilli per gram tissue of eight mice.

Bifidobacterium bifidum grows only in tumor tissues.

Kimura NT, et al., Cancer Res, 1980, 40, 2061
**B. bifidum** (Lac B) & lactulose

Lactulose: A synthesized disaccharide from lactose. It is not metabolized by mammalian tissue cells.

Lactulose *in vivo* stimulates the growth of *B. bifidum* in the tumor.

The non-pathogenicity of *B. bifidum* (Lac B)

- The animals expressed no visible adverse symptoms.
- It was confirmed that *B. bifidum* is safe bacteria injected intravenously.

### Anaerobic bacteria characteristics

Three classes of anaerobic and facultative anaerobes have been tested for anticancer therapy:

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Features</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class I:</strong></td>
<td>B. longum</td>
<td>Gram⁺, facultative anaerobes</td>
<td>Non-pathogenic present in common intestinal flora, Have been used in human for many years</td>
<td>No obvious oncolytic effect</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>B. adolescentis</td>
<td>Obligate anaerobes</td>
<td>Probiotic bacteria, Can be used for intravenous or oral administration</td>
<td>Non-spore former</td>
</tr>
<tr>
<td></td>
<td>B. infantis</td>
<td></td>
<td>Expression of recombinant protein</td>
<td>More susceptible to non-permissive conditions</td>
</tr>
<tr>
<td><strong>Class II:</strong></td>
<td><em>Salmonella</em></td>
<td>Gram⁻, facultative anaerobes</td>
<td>Attenuated vaccine strain has been proved safe clinically in human, Biochemistry pathways and genomes are well characterized</td>
<td>Intracellular bacteria, thus may have difficulty to infect and lyse quiescent cell</td>
</tr>
<tr>
<td>Faculative</td>
<td><em>S. typhimurium</em></td>
<td></td>
<td>Tumours have intrinsic antitumour activity</td>
<td>Have a tumour to normal tissue ratio of 1000:1, therefore a significant number of bacteria colonize normal organs</td>
</tr>
<tr>
<td>intracellular</td>
<td><em>S. choleraeis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td><em>Listeria</em></td>
<td>Gram⁺, facultative anaerobes</td>
<td>Grow under aerobic and anaerobic conditions, thus can target both large and small tumours, enter professional antigen presenting cells and induce strong innate immune response</td>
<td>Virulence factors exist, especially LPS in the bacterial cell wall, thus safety is an issue when large amount of bacteria are delivered</td>
</tr>
<tr>
<td></td>
<td><em>L. monocytogenes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Class III:</strong></td>
<td><em>Clostridium</em></td>
<td>Gram⁻, strictly anaerobes</td>
<td>Spore former, Spores are stable, easy to produce and economic to use</td>
<td>Some strains are pathogenic, Some strains are difficult to manipulate genetically</td>
</tr>
<tr>
<td>Strictly</td>
<td><em>Proteolytic</em></td>
<td>normal habitat in the soil, aquatic sediments, and intestinal tract of both animals and humans</td>
<td>Clostridial spores can be delivered non-invasively and systemically, i.e. intravenous injection</td>
<td>Only colonize in large tumours with area of hypoxia/necrosis</td>
</tr>
<tr>
<td>Anaerobic</td>
<td><em>C. sporogenes</em></td>
<td></td>
<td></td>
<td>Oncolyis interrupted at the rim causing incomplete tumour lysis</td>
</tr>
<tr>
<td>bacteria</td>
<td><em>Saccharolytic</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. nosi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. butyricum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. acetobutylicum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. oncolyticum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. beijerinckii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M.Q Wei et al., Cancer Letters, 2008, 259, 16
Summary of Anaerobic bacteria characteristics

- Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells.
  - *Bifidobacterium* is non-pathogenic, but it doesn’t show oncolytic effect.
  - *Clostridium* extensive oncolytic effect, but some strains are pathogenic.

- Facultative anaerobic bacteria shows pathogenic or toxic effect on both tumor cells and normal cells.
  - *Streptococcus, Salmonella, Listeria, E. coli*, etc...
Summary of bacterial therapy for cancer

• The original bacterial therapy for cancer was performed over 300 years ago.

• Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells.

• Obligate anaerobic bacteria (Clostridium and Bifidobacterium) treatment alone was not enough to control tumor significantly.
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Bifidobacterium used for a novel DDS

- *Bifidobacterium* are non-invasive bacteria.
- Expression vector insertion enables to carry the desired peptides and proteins into the solid tumors.
Examples of *Bifidobacterium* DDSs

- Anti-PD-1 antibody scFv producing *B. longum*

- Trastuzumab scFv producing *B. longum*

5-Fluorouracil (5-FU)

• **5-FU**
  - a medication used to treat cancer.
  - a thymidylate synthase (TS) inhibitor.

• **Mechanism**
  - 5-FU inhibits Thymidylate synthase resulting in impaired DNA synthesis.
Cytosine deaminase (CD)

- Cytosine deaminase (CD)
  - CD converts low-toxic 5-fluorocytosine (5FC) to active 5-fluorouracil (5FU)

\[
\text{5-FC} \xrightarrow{\text{Cytosine deaminase (CD)}} \text{5-FU}
\]

- The cytosine deaminase of *Escherichia coli* (e-CD) was inserted into the plasmid under the promoter region of the plasmid.
CD producing *B. longum*

- **Concept**

![Diagram of CD producing B. longum](image)

- 5-FC is produced and activated in tumor cells only.

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**Fig. 2.** Concept of cancer treatment by combining cytosine deaminase of *Escherichia coli* (e-CD)-transformed *Bifidobacterium longum* (i.v.) with the prodrug 5-fluorocytosine (5FC) (given orally). 5FU, 5-fluorouracil.
Suppression of tumor growth was observed in the groups treated with i.v. injection of the bacteria and the prodrug 5-FC given orally.

Sasaki T, et al., Cancer Sci., 2006, 97, 649
Anaeropharma Science

- Anaeropharma Science was established in Tokyo, Japan in 2004.

- The Phase I/II trial of APS001F is currently ongoing in US.

- This company works on a joint development project with *Eisai Co., Ltd.* and *Astellas Pharma Inc.* respectively.
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• Obligate anaerobic bacteria intravenously injected accumulates only in tumor cells because of its anaerobic condition.

• Obligate anaerobic bacteria (*Clostridium* and *Bifidobacterium*) treatment alone was not enough to control tumor significantly.

• *Bifidobacterium* is being used to treat cancer as a novel DDS, or a safety carrier of the therapeutic proteins.
Clinical trial of *Salmonella* VNP20009

### Table 1. Characteristics of Patients Receiving VNP20009

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of Patients (N = 25)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

Results of counting No. of bacteria in tumors

FNA: fine needle aspiration cytology

### Table 3. Tumor Biopsy Cultures After the Administration of VNP20009

<table>
<thead>
<tr>
<th>Dose (dV/m^2)</th>
<th>Patient No.</th>
<th>Tumor Biopsy</th>
<th>Day</th>
<th>cv/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10^6</td>
<td>1</td>
<td>FNA, excised</td>
<td>5, 14</td>
<td>0</td>
</tr>
<tr>
<td>3 x 10^6</td>
<td>2</td>
<td>FNA</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>3</td>
<td>FNA</td>
<td>2, 15, 35</td>
<td>0</td>
</tr>
<tr>
<td>3 x 10^6</td>
<td>4</td>
<td>FNA</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>7(1)</td>
<td>FNA</td>
<td>3, 15</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>8</td>
<td>FNA</td>
<td>2, 16</td>
<td>0</td>
</tr>
<tr>
<td>3 x 10^6</td>
<td>10</td>
<td>FNA</td>
<td>2, 13, 18</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>12</td>
<td>FNA</td>
<td>2, 16</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>13(2)</td>
<td>FNA, excised</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>14</td>
<td>Excised, FNA</td>
<td>6, 15</td>
<td>0</td>
</tr>
<tr>
<td>3 x 10^6</td>
<td>15</td>
<td>FNA</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>23</td>
<td>FNA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>24</td>
<td>FNA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3 x 10^6</td>
<td>25</td>
<td>FNA (rt lower leg)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^9</td>
<td>19</td>
<td>FNA (rt lower leg)</td>
<td>4</td>
<td>11,000</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>20</td>
<td>FNA (rt upper leg)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>21</td>
<td>FNA (liver)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10^8</td>
<td>22</td>
<td>FNA (liver)</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Abbreviations: rt, right; ant, anterior; lat, lateral. *Number in parentheses indicates the patient’s treatment cycle.