Bladder Cancer

• Bladder cancer genesis
  ✓ Con A agglutination assay
  ✓ Promoting activity of leucine and isoleucine
  ✓ Hypersusceptibility of bladder cancer in analbuminemic rats

• Relation between papillary and nodular carcinoma of the bladder

• Ileal neobladder to ensure normal urination after cystectomy
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Isolation of Epithelial Cells from Rat Bladder

1. Remove bladder
2. Evert
3. Saline with 5 mM EDTA
4. Stand for 15 min
5. Ultrasonic cuvette washer 5 sec

Isolated cells
Con A Agglutination Assay

Con A 200μg/ml

0.5~1.2 × 10^6 cells/ml

± α-MM

Shake at 37°C for 30 min

PBS

Count in haemocytometer
Appearance of Con A Agglutination of Bladder Cells after Administration of BHBM
Short-term Assay of Promoters of Bladder Cancer
Maintenance by DL-Tryptophan of Increased Agglutinability Caused by BHBN
Maintenance by Amino acids of Increased Agglutinability of Bladder Cells by Con A

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Agglutination*</th>
<th>Amino acid</th>
<th>Agglutination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>3 ± 1</td>
<td>L-Lysine</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>4 ± 1</td>
<td>L-Methionine</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>4 ± 1</td>
<td>DL-Phenylalanine</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>3 ± 1</td>
<td>L-Phenylalanine</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>4 ± 1</td>
<td>L-Proline</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>6 ± 2</td>
<td>L-Serine</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>8 ± 2</td>
<td>L-Threonine</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>6 ± 1</td>
<td>DL-Tryptophan</td>
<td>20 ± 3**</td>
</tr>
<tr>
<td>DL-Histidine</td>
<td>6 ± 2</td>
<td>D-Tryptophan</td>
<td>20 ± 2**</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>5 ± 2</td>
<td>L-Tryptophan</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>L-Hydroxyproline</td>
<td>4 ± 2</td>
<td>L-Tyrosine</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>24 ± 2**</td>
<td>D-Valine</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>D-Leucine</td>
<td>6 ± 1</td>
<td>L-Valine</td>
<td>14 ± 2**</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>19 ± 4**</td>
<td>0.01% BHBN alone</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

* mean ± standard deviation  ** P < 0.001
Chemical Structure of Leucine, Isoleucine and Valine
Long-term Experiment on Promoting Effect of L-Isoleucine and L-Leucine in Rat Bladder Carcinogenesis
Histological Findings in the Urinary Bladder of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective No. of animals</th>
<th>Incidence (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PN hyperplasia</td>
<td>Papilloma</td>
<td>Carcinoma</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>BHBN→2% Ile</td>
<td>31</td>
<td>25 (81)</td>
<td>18 (58)</td>
<td>14 (45)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BHBN→4% Ile</td>
<td>31</td>
<td>22 (71)</td>
<td>19 (61)</td>
<td>24 (77) ***</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BHBN→2% Leu</td>
<td>31</td>
<td>23 (74)</td>
<td>15 (48)</td>
<td>16 (52) **</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BHBN→4% Leu</td>
<td>31</td>
<td>20 (64)</td>
<td>16 (52)</td>
<td>23 (74) ***</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BHBN</td>
<td>31</td>
<td>21 (68)</td>
<td>21 (68)</td>
<td>7 (23)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4% Ile</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4% Leu</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**p < 0.01, ***p < 0.001
Age-adjusted Mortality Rate for Bladder Cancer by per Capita Protein Consumption

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Polyacrylamide Gel Electrophoresis of Serum Proteins
Experimental Schedule

**Analbuminemic rats** \( \sigma^\prime \), **8-wk-old**

- **A)**
  - 0
  - 3wk
  - 8wk
  - 20wk
  - 0.045% BHBN
  - 0.05% BHBN

**Sprague-Dawley rats** \( \sigma^\prime \), **8-wk-old**

- **B)**
  - 0
  - 20wk

- **C)**
  - 0
  - 8wk
  - 20wk
  - 0.05% BHBN

- **D)**
  - 0
  - 20wk
Change of Body Weight
Intake of BHBN

Body weight (g) vs. BHBN for different groups:
- Analbuminemic rats - BHBN
- Analbuminemic rats
- Sprague-Dawley rats - BHBN
- Sprague-Dawley rats
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Papillary Carcinoma
Nodular Carcinoma
Papillonodular Carcinoma
CIS

Sever and mild dysplasia
Bladder Carcinogens for Animal Experiment

N-Butyl-N-(4-hydroxybutyl) nitrosamine
(BHBN)
\[ \text{ON} - \text{N} - \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]
\[ \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH} \]

N-[4-(5-Nitro-2-furyl)-2-thiazolyl] formamide
(FANFT)

N-Methly-N-nitrosourea
(MNU)
\[ \text{ON} - \text{N} - \text{CH}_3 \]
\[ \text{C} - \text{NH}_2 \]
Bladder Carcinogenesis in Rats

Normal  Simple hyperplasia  PN hyperplasia  Papilloma  Carcinoma
Bladder Carcinogenesis in Mice

Normal → Simple hyperplasia → Dysplasia → CIS → Carcinoma
Bladder Carcinogenesis in Dogs

BHBN 80mg/day, 4-5 year

BHBN 500mg/day, 1 year

# Naturally Occurring Bladder Cancer

<table>
<thead>
<tr>
<th>Institution</th>
<th>Case</th>
<th>Av.age</th>
<th>Tumor type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Cancer Center and Osaka Medical Center for Cancer and Cardiovascular Diseases</td>
<td>1,910</td>
<td>60</td>
<td>70</td>
</tr>
</tbody>
</table>
Relation between papillary and nodular carcinoma

- Analysis by step section of cystectomized specimens
Subjects


All underwent cystectomy and the specimens were examined by step-sectioning.

23—83 y.o. (mean 60.8 y.o.)

Male to female ratio 155 to 48 (3:1)

Composition of Bladder Cancer
Coexistence of Papillary and Nodular Carcinomas in the Bladder: pT1 Cases.

- Blue: Papillary carcinoma
- Yellow: Papillonodular carcinoma
- Pink: Nodular carcinoma
Coexistence of Papillary and Nodular Carcinomas in the Bladder: pT2 Cases
Coexistence of Papillary and Nodular Carcinomas in the Bladder: pT3 Cases
Coexistence of Papillary and Nodular Carcinomas in the Bladder: pT4 Cases
Coexistence of Papillary and Nodular Carcinomas in the Bladder: Cases Having History of Papillary Carcinomas
## Papillary Structures in Nodular Carcinoma

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. of Pt</th>
<th>Papillary structure (+)</th>
<th>Papillary structure (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P + N / P+N+C</td>
<td>33</td>
<td>32 (97*)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>N / N+C</td>
<td>55</td>
<td>29 (53)</td>
<td>26 (47)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage
Conceptual Course of Development of Bladder Cancer

Hyperplasia dysplasia → P → P → P → P±C

N±C → C

P, PN → P, PN, N±C

P, PN, N, ±C
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Multicentric Development of Transitional Cell Carcinomas in the Urinary Tract

- 20~58%
- 15~50%
- 4~17%
- 50~80%
TCC of the urethra in Men Following Cystectomy for Bladder Cancer: Multivariate Analysis for Risk Factors


Significant risk factors were
• Papillary cancer
• Multiple cancer
• Tumor in the bladder neck, prostatic urethra and prostatic gland
## Risk Factors and Urethral Recurrence

<table>
<thead>
<tr>
<th>No. risk factors</th>
<th>No. cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0/46 (0%)</td>
</tr>
<tr>
<td>1</td>
<td>2/44 (4.5%)</td>
</tr>
<tr>
<td>2</td>
<td>6/46 (13%)</td>
</tr>
<tr>
<td>3-4</td>
<td>10/33 (30%)</td>
</tr>
</tbody>
</table>
Urethral Involvement in Female Bladder Cancer Patients: Mapping of 47 Consecutive Cysto-urethrectomy Specimens

P. Coloby, T. Kakizoe et al. J. Urol 1994

Risk factors were

• Papillary or papillonodular carcinoma encroaching on the bladder neck
• Nodular invasive carcinoma infiltrating the bladder neck and trigone
Three Cases of Female Bladder Cancer Involving the Urethra
An ileal neobladder for a female patient after a radical cystectomy to ensure voiding from the urethra: A case report