Novel Dual Cancer Stem Cell and Telomerase Targeting for the Treatment of Colorectal Cancer

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Disclosure

• S. Steve Chung has no relevant financial relationship to disclose.
Grant Supports

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**U54 MD 007598**
Accelerating Excellence in Translational Science (AXIS)

**R25 MD 007610**
Clinical Research Education Career Development (CRECD)

**S21 MD 000103**
Emerging Scientist Award
Learning objectives

• Upon completion of this session, participants will be able to:
  • Describe how pro-inflammatory cytokines promote cancer cells to stem-like cell transition.
  • Recognize how withaferin A can impact clinical practices for metastatic colorectal cancer.
### Estimated New Cases

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>220,800</td>
<td>26%</td>
<td>Breast</td>
<td>231,840</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>115,610</td>
<td>14%</td>
<td>Lung &amp; bronchus</td>
<td>105,590</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>69,090</td>
<td>8%</td>
<td>Colon &amp; rectum</td>
<td>63,610</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>56,320</td>
<td>7%</td>
<td>Uterine corpus</td>
<td>54,870</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>42,670</td>
<td>5%</td>
<td>Thyroid</td>
<td>47,230</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>39,850</td>
<td>5%</td>
<td>Non-Hodgkin lymphoma</td>
<td>32,000</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>38,270</td>
<td>5%</td>
<td>Melanoma of the skin</td>
<td>31,200</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>32,670</td>
<td>4%</td>
<td>Pancreas</td>
<td>24,120</td>
</tr>
<tr>
<td>Leukemia</td>
<td>30,900</td>
<td>4%</td>
<td>Leukemia</td>
<td>23,370</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>25,510</td>
<td>3%</td>
<td>Kidney &amp; renal pelvis</td>
<td>23,290</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>848,200</strong></td>
<td><strong>100%</strong></td>
<td><strong>All Sites</strong></td>
<td><strong>810,170</strong></td>
</tr>
</tbody>
</table>

### Estimated Deaths

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>86,380</td>
<td>28%</td>
<td>Lung &amp; bronchus</td>
<td>71,660</td>
</tr>
<tr>
<td>Prostate</td>
<td>27,540</td>
<td>9%</td>
<td>Breast</td>
<td>40,290</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,110</td>
<td>8%</td>
<td>Colon &amp; rectum</td>
<td>23,600</td>
</tr>
<tr>
<td>Pancreas</td>
<td>20,710</td>
<td>7%</td>
<td>Pancreas</td>
<td>19,850</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>17,030</td>
<td>5%</td>
<td>Ovary</td>
<td>14,180</td>
</tr>
<tr>
<td>Leukemia</td>
<td>14,210</td>
<td>5%</td>
<td>Leukemia</td>
<td>10,240</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,600</td>
<td>4%</td>
<td>Uterine corpus</td>
<td>10,170</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>11,510</td>
<td>4%</td>
<td>Non-Hodgkin lymphoma</td>
<td>8,310</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>11,480</td>
<td>4%</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>7,520</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>9,070</td>
<td>3%</td>
<td>Brain &amp; other nervous system</td>
<td>6,380</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>312,150</strong></td>
<td><strong>100%</strong></td>
<td><strong>All Sites</strong></td>
<td><strong>277,280</strong></td>
</tr>
</tbody>
</table>

**FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2015.**

Estimates are rounded to the nearest 10 and cases exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.
- Regarding health disparity, it is critical to understand the complex interactions that exist between environmental factors such as diet, lifestyle and living conditions, and genetic susceptibility to cancer risk.
- Risk is high because of *excessive intakes of animal meat and fat products* and *differences in colonic bacterial metabolism*.

- The development of greater *tumor virulence* possibly resulting from *disparities in insurance status, screening behaviour, treatment patterns, social support, and access to and use of health care facilities*.

Our Mission

In 2002, the Department of Internal Medicine at CDU established a formal Division of Cancer Research and Training under the leadership of Dr. Jay Vadgama. Our mission is to design and conduct basic, clinical, applied, translational, and prevention research programs directed toward reducing cancer incidence, morbidity, and mortality in the underserved and minority communities which face cancer health disparities in Service Planning Area (SPA) 6 region of Los Angeles.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>SPA 6</th>
<th>L.A. County</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer deaths (per 100,000)</td>
<td>209.0</td>
<td>164.5</td>
</tr>
<tr>
<td>Lung cancer death rate (per 100,000)</td>
<td>46.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Breast cancer death rate among females (per 100,000)</td>
<td>27.8</td>
<td>23.1</td>
</tr>
<tr>
<td>Colorectal cancer death rate (per 100,000)</td>
<td>23.2</td>
<td>16.2</td>
</tr>
<tr>
<td>Percent of adults ages 18-64 without health insurance</td>
<td>31.7</td>
<td>21.8</td>
</tr>
<tr>
<td>Percent of population living below federal poverty line</td>
<td>28.0</td>
<td>16.2</td>
</tr>
</tbody>
</table>
CDU/UCLA Cancer Center Partnership Renewal Awarded $12.6 Million

The Charles R. Drew University of Medicine and Science (CDU)/UCLA Cancer Center Partnership to Eliminate Cancer Health Disparities has been awarded over $12.6M from the National Institutes of Health (NIH) and the National Cancer Institute (NCI).

Under the leadership of the Principal Investigator, Dr. Jay Vadgama, CDU will receive over $9M. An additional $3.6M has been awarded to the University’s longtime partner, the Jonsson Comprehensive Cancer Center (JCCC) at the David Geffen School of Medicine at UCLA under the leadership of Dr. Robin Farias-Eisner. The Partnership will receive $12.6M, over the next 5 years, to achieve its mission to reduce cancer health disparities in South Los Angeles.

“Our Partnership will continue to build on our capability to reduce cancer health disparities among underserved populations,” said Dr. Jay Vadgama, who is also Vice President.
Colorectal cancer metastasis

- 25 ~35% of patients present with synchronous metastasis.
- 50 ~ 60% of patients will eventually develop metastasis, mostly within 2 years of detecting primary.
Gaps between the goal and what we know

- Molecular mechanisms by which pro-inflammatory cytokines leading to **metastatic CRC**.
- **Specific stimulation** which elicit transitional signaling that leads to invasive phenotype in CRC.
- **Efficient inhibitors** for CRC activation signaling to target the cancer stem cell population and telomerase.
Inflammation and cancer

• Inflammation has been proposed to mediate the initiation and promotion of tumors, angiogenesis, and metastasis (Grivennikov and others 2010).

• Several cytokines regulate the inflammatory tumor microenvironment.

• Herein, we explored two cytokines (IL6 and TNFa) for their ability to promote cancer metastasis.
IL-6

• Elevated expression of IL-6, which can be detected in patient serum, is linked to increased risk of development of colorectal adenomas.

• Serum studies of cancer patients suggested IL-6 may reflect prognosis and tumor load.

→ Elevated IL-6 levels have been associated with advanced stage and metastasis-related morbidity.
In a series of 122 patients with renal cell carcinoma, it was demonstrated that serum levels of TNF-α directly correlated with advanced stage grouping as compared with controls, and suggested that TNF-α could be useful in the early diagnosis of the disease.
CONCLUSION: Serum IL-6, TNFα and CRP levels definitely increase in CRC patients. Pre-operative serum elevation of IL-6 and CRP was thus found to be predictor of the prognosis of CRC patients. The clinical value of TNFα in CRC needs to be further investigated.

C-reactive protein (CRP), a protein synthesized in the hepatocytes, has also been reported to be related both to the malignant potential of the neoplasms and to physical cachexia.
Coexpression of IL-6 and TNF-α: prognostic significance on breast cancer outcome

Figure 1. Overall survival of breast cancer patients according to the coexpression of IL-6 and TNF-α (Group A: patients with both IL-6 and TNF-α low; Group B: patients with IL-6 or TNF-α high; Group C: patients with both IL-6 and TNF-α high). Log-rank test: p=0.168 (Group A vs Group B); p=0.002 (Group A vs Group C); p=0.035 (Group B vs Group C).

cut-off points to subdivide breast cancer patients into (i) patients with low or high IL-6 (if IL-6 was <6.81 pg/ml or ≥6.81 pg/ml, respectively) and (ii) patients with low or high TNF-α (if TNF-α was <18.93 pg/ml or ≥18.93 pg/ml, respectively).
Serum levels of IL-6 and TNF-α correlate with clinicopathological features and patient survival in patients with prostate cancer.

With recurrent prostate cancer, serum IL-6 levels in patients with metastatic disease (9.3 ± 7.8 pg ml⁻¹) were higher than those in patients with localised disease (1.3 ± 0.8 pg ml⁻¹, P < 0.001). Significantly elevated levels of TNF-α were found in metastatic disease (6.3 ± 3.6 pg ml⁻¹) compared with localised disease (1.1 ± 0.5 pg ml⁻¹, P < 0.001). The levels of both cytokines were directly correlated with the extent of the disease. Serial analysis in 40 patients with recurrent tumours showed that both cytokines became
Serum Level of Interleukin 6 & Tumor Necrosis Factor in Iraqi Breast Cancer patients

Table (1) Serum IL-6 and TNF-α levels in breast cancer patients at different clinical stages

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>No, samples</th>
<th>IL-6 mean ± SD pg / ml</th>
<th>TNF-α mean ± SD pg / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>15.3 ± 6.1</td>
<td>27.5 ± 5.1</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>30 ± 7.1</td>
<td>50 ± 6.2</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>34 ± 10.1</td>
<td>49 ± 5.2</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>40 ± 5.2</td>
<td>66 ± 6.1</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>5.1 ± 4.9</td>
<td>4.5 ± 4.8</td>
</tr>
</tbody>
</table>

* LSD = Least Significant Value  
** S = Significant between the groups  
*** r = Correlation coefficient

Table (2) Serum IL-6 and TNF α levels in breast cancer patients with different metastasis sites

<table>
<thead>
<tr>
<th>Site of Metastasis</th>
<th>No. samples</th>
<th>IL-6 mean ± SD pg / ml</th>
<th>TNF α mean ± SD pg / ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td>17</td>
<td>25.4 ± 0.3</td>
<td>41 ± 0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>60 ± 5.1</td>
<td>51 ± 2.1</td>
</tr>
<tr>
<td>Bone</td>
<td>8</td>
<td>50 ± 3.2</td>
<td>30.1 ± 4.2</td>
</tr>
<tr>
<td>Numerous</td>
<td>11</td>
<td>67 ± 5.3</td>
<td>65 ± 1.2</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Metastatic variant = Cancer Stem Cells

- Pro-inflammatory cytokines, IL6 and TNF-α, treatments $\rightarrow$ Metastasis
- Metastatic variant cells are considered cancer stem cells.
- In this study, we tried to stimulate colorectal cancer cells with IL-6 and TNF-α to induce them more aggressive, stem-like and metastatic.
Cancer Stem Cell [CSC] Characteristics

- Minor population in tumor: 0.1 - a few percent
- Self-renewing; infinite proliferative potential.
- Enhanced resistance to drugs, radiation, cell stress.
- Tumorigenic; give rise to other cell types in tumor.
- Associated with metastasis and relapse.

Metastasis and relapse are involved in more than 90% of all cancer deaths.

Strategies to eradicate CSCs are an urgent topic in cancer research.

<table>
<thead>
<tr>
<th>STEM CELL MARKER</th>
<th>TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34/CD38</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>CD44/CD24+/ALDH</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Side Population (SP)</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>CD133, CD44, EpCAM, ALDH</td>
<td>Colorectal carcinoma</td>
</tr>
<tr>
<td>CD133, Side Population (SP)</td>
<td>Endometrial carcinoma</td>
</tr>
<tr>
<td>CD133, CD44</td>
<td>Ewing’s sarcoma</td>
</tr>
<tr>
<td>CD44</td>
<td>Gastric carcinoma</td>
</tr>
<tr>
<td>CD44, Side Population (SP), ALDH</td>
<td>Head and neck carcinoma</td>
</tr>
<tr>
<td>CD133, CD44, ALDH</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>CD133, Side Population (SP), ALDH</td>
<td>Lung carcinomas (non-small cell and small cell)</td>
</tr>
<tr>
<td>CD133</td>
<td>Medulloblastoma, Glioma</td>
</tr>
<tr>
<td>CD133, CD44, CD24, ALDH, EpCAM</td>
<td>Pancreatic carcinoma</td>
</tr>
<tr>
<td>CD133, CD44, ALDH</td>
<td>Prostate carcinoma</td>
</tr>
</tbody>
</table>

Cancer Stem Cells - The Cutting Edge, Edited by Stanley Shostak, 2011
• **HT-29** is a human colorectal adenocarcinoma cell line with epithelial morphology.

• established in 1964 from the primary tumor of a 44-year-old Caucasian female with colorectal adenocarcinoma.
Combined treatments of IL6 and TNFα activated STAT3 synergistically

<table>
<thead>
<tr>
<th></th>
<th>DLD1</th>
<th>HT-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>pSTAT3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STAT3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pNF-kB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(p65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Time (hr) 0 6 12 24

**pSTAT3/STAT3 ratio**

- UT
- IL6
- TNF-α
- IL6+TNFα

**pNF-kB/β-actin ratio**

- UT
- IL6
- TNF-α
- IL6+TNFα

Combined treatments of IL6 and TNFα activated STAT3 synergistically.
Withaferin A

- A steroidal lactone that is isolated from the plant *Withania somnifera* (*ashwagandha*).
- It has potent anti-inflammatory properties.
- In recent years, it has been suggested as a potential anti-cancer compound shown to prevent tumor growth, angiogenesis, and metastasis in various types of cancer.
Purpose

Withania somnifera (WSE; Ashwagandha in Ayurveda) extracts have been used as an adaptogen or to build resistance to stress or diseases in indigenous medical systems in India for centuries. Modern scientific data for WSE indicate several bioactive molecules (withanolides, withanosides, indoles, withaferin-A, others) with significant immunomodulatory, anti-inflammatory and stress reducing properties.

This study will examine whether a standardized extract of Withania Somnifera (WSE; Sensoril®) will improve total, positive, negative symptoms, and stress in patients with schizophrenia. The study will examine whether WSE reduces PANSS positive and negative symptoms and stress scores in subjects, and whether these improvements are mediated by changes in inflammatory immune indices. An additional aim will determine if patients receiving WSE will have fewer adjustments to their psychotropic medications that those assigned to placebo. The study will examine whether WSE will re-balance Th1/Th2 ratios (cytokine measures) and mediate a reduction of elevated hs-CRP levels. It is hypothesized that those subjects whose Th1/Th2 ratios normalize will likely have a greater magnitude of clinical improvement versus those subjects whose immune ratios remain unbalanced.

The proposal is a 12-week, double-blind, placebo-controlled RCT of WSE added to antipsychotic medications in approximately 60 or more patients with schizophrenia with an exacerbation of symptoms. If efficacy is affirmed, this low cost extract could be studied further, and used quite readily across, low, middle and high income countries.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia</td>
<td>Drug: Sensoril®</td>
</tr>
<tr>
<td>Schizoaffective Disorder</td>
<td>Drug: Placebo</td>
</tr>
</tbody>
</table>

Study Type: Interventional

Study Design: Allocation: Randomized
Intervention Model: Parallel Assignment
Masking: Participant, Care Provider, Investigator, Outcomes Assessor
Primary Purpose: Treatment

Official Title: Sensoril® (Ashwagandha), an Immunomodulator and Anti-inflammatory Agent for Schizophrenia: A Parallel Group, Randomized Double Blind, and Placebo Controlled Study
**Withaferin A inhibited STAT3 activation and Oct-4 expression**

<table>
<thead>
<tr>
<th></th>
<th>DLD1</th>
<th>HT-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pSTAT3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pNF-kB</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oct-4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-actin</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- IL-6: Inhibitory effect
- TNF-α: Activating effect
- WA: Effect not determined
- pSTAT3: Phosphorylated STAT3
- pNF-kB: Phosphorylated NF-kB
- Oct-4: Octamer-binding transcription factor
- β-actin: Loading control

Withaferin A inhibited STAT3 activation and Oct-4 expression, as indicated by the + symbol for pSTAT3 and Oct-4 in both DLD1 and HT-29 cells. The Western blot results show a lack of pNF-kB and β-actin expression, indicating no effect on these pathways.
Oct-4

• Tumor dedifferentiation is a well known phenomenon and it has long been proposed to be involved in tumor progression (Gabbert et al. 1985).
• Similar to somatic cell reprogramming, tumor dedifferentiation is reversal of cell development to a more immature state.
• Oct4 is the most critical transcription factor since it can reprogram adult stem cells to iPS cells as a single factor (Kim et al. 2009).
• Tumorigenesis and somatic cell reprogramming share common mechanisms (Daley 2008).
1. Stem cell

   Stem Cell

   Normal stem cell

2. Progenitor cell

   Mutated stem cell, or self-renewal genes turned on

   Normal progenitor cell

   Mutated progenitor, or self-renewal genes turned on

3. Differentiated cell

   Loss of regulated cell division

   Self-renewal genes turned on

   Cancer stem cell

Source: NIH
Aberrant expression of *Oct4, Nanog, Sox2, Lin28 and Klf4* are all associated with abnormal tissue growth or tumorigenesis.
3D Spheroid colony formation assay using human breast cancer; enrichment of cancer stem cells

Transwell cell migration assay
Sphere formation and trans-well invasion studies

A

Methanol
IL6 + TNFα
IL6+TNFα +WA

B

Colonosphere formation (spheres/well)

Methanol
IL6 + TNFα
IL6+TNFα +WA

C

Methanol
IL6 + TNFα
IL6+TNFα +WA

D

Invaded cells Percentile

Methanol
IL6+TNFα+WA
IL6 + TNFα

* p < 0.05
** p < 0.01
**STAT3-NF-κB** protein-protein interactions in cancer stem cell

Co-Immunoprecipitation assay for protein-protein interactions

Cell Lysate or Protein Mixture

Incubation with Antibody-coupled Resin

Spin and wash

Elute

Coupled Antibody

Statistic 3

Antigen

Protein Interacting with Antigen

NF-kB

Analyze
**IL6/TNFα treatments induced STAT3-NF-kB complex formation, Withaferin A abolished the interactions**

---

**DLD-1**

**A.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NF-kB (p65)</th>
<th>STAT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + IL6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ + + TNF-α</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Immunoblot**

- IgG
- STAT3

**B.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NF-kB Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 - TNF-α WA</td>
<td>STAT3 Immunoprecipitate 16 hours at 4°C</td>
</tr>
</tbody>
</table>

**HT-29**

**C.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NF-kB (p65)</th>
<th>STAT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + + IL6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+ + + TNF-α</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Immunoblot**

- IgG
- STAT3

**D.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NF-kB Immunoblot</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 - TNF-α WA</td>
<td>STAT3 Immunoprecipitate 16 hours at 4°C</td>
</tr>
</tbody>
</table>

- WA: Withaferin A
Telomerase

A ribonucleoprotein that adds the polynucleotide "TTAGGG" to the 3' end of telomeres, which are found at the ends of eukaryotic chromosomes.

Telomere

A region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes.
Telomerase modulates Wnt signalling by association with target gene chromatin

Jae-II Park¹, Andrew S. Venteicher¹,²*, Ji Yeon Hong⁴*, Jinkuk Choi¹,³, Sohee Jun¹, Marina Shkreli¹, Woody Chang¹, Zhaojing Meng⁵, Peggy Cheung¹, Hong Ji⁴, Margaret McLaughlin⁶, Timothy D. Veenstra⁵, Roel Nusse⁷, Pierre D. McCrea⁴ & Steven E. Artandi¹,²,³

Stem cells are controlled, in part, by genetic pathways frequently dysregulated during human tumorigenesis. Either stimulation of Wnt/β-catenin signalling or overexpression of telomerase is sufficient to activate quiescent epidermal stem cells in vivo, although the mechanisms by which telomerase exerts these effects are not understood. Here we show that telomerase directly modulates Wnt/β-catenin signalling by serving as a cofactor in a β-catenin transcriptional complex. The telomerase protein component TERT (telomerase reverse transcriptase) interacts with BRG1 (also called SMARCA4), a SWI/SNF-related chromatin remodelling protein, and activates Wnt-dependent reporters in cultured cells and in vivo. TERT serves an essential role in formation of the anterior–posterior axis in Xenopus laevis embryos, and this defect in Wnt signalling manifests as homeotic transformations in the vertebrae of Tert⁻/⁻ mice. Chromatin immunoprecipitation of the endogenous TERT protein from mouse gastrointestinal tract shows that TERT physically occupies gene promoters of Wnt-dependent genes. These data reveal an unanticipated role for telomerase as a transcriptional modulator of the Wnt/β-catenin signalling pathway.
Telomerase, the repetitive sequences at chromosomal ends, protect intact chromosomes. Telomeres progressively shorten through successive rounds of cell divisions, and critically shortened telomeres trigger senescence and apoptosis. The enzyme that elongates telomeres and maintains their structure is known as telomerase. The catalytic subunit of this enzyme (telomerase reverse transcriptase [TERT]) is expressed at a high level in malignant cells, but at a very low level in normal cells. Although telomerase activity was long believed to be the only function of TERT, emerging evidence indicates that TERT plays roles beyond telomeres. For example, TERT contributes to stem cell maintenance and cell reprogramming processes in a manner independent of its canonical function. Even some types of splice variants that lack the telomerase catalytic domains exhibit the functions in a manner that does not depend on telomerase activity. We recently demonstrated that the RNA-dependent RNA polymerase (RdRP) activity of TERT is involved in regulation of gene silencing and heterochromatic transcription. Moreover, TERT RdRP activity is mediated by a newly identified complex, distinct from the authentic telomerase complex, that plays a role in cancer stem cells in a telomere maintenance independent manner. TERT has attracted interest as a molecular target for anticancer treatment, but previous efforts aimed at developing novel therapeutic strategies focused only on the canonical function of TERT. However, accumulating evidence about the non-canonical functions of TERT led us to speculate that the functions other than telomerase might be therapeutic targets as well. In this review, we discuss the non-canonical functions of TERT and their potential applications for anticancer treatment.
Chromatin Immunoprecipitation (ChIP assay)
STAT3 binding sites

A.

B.

C.

D.

STAT3 bound on the hTERT promoter region
telomerase PCR-ELISA kit (TeloTAGGG Telomerase PCR ELISA, Roche)
Cytokine increased telomerase activity whereas withaferin A reduced it

E. Telomerase activity (2 X 10⁵ cells)

<table>
<thead>
<tr>
<th>Condition</th>
<th>O.D. 650 – O.D. 750</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>2</td>
</tr>
<tr>
<td>IL6</td>
<td>2</td>
</tr>
<tr>
<td>TNF-α</td>
<td>2</td>
</tr>
<tr>
<td>IL6 + TNF-α</td>
<td>3</td>
</tr>
<tr>
<td>IL6 + TNF-α + WA</td>
<td>1</td>
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F. Telomerase activity (1 X 10⁴ cells)

<table>
<thead>
<tr>
<th>Condition</th>
<th>O.D. 650 – O.D. 750</th>
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<tbody>
<tr>
<td>Untreated</td>
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<tr>
<td>IL6</td>
<td>0.7</td>
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<tr>
<td>TNF-α</td>
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<td>IL6 + TNF-α</td>
<td>0.9</td>
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<td>IL6 + TNF-α + WA</td>
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</table>
1 EGFR/ErbB1
2 HER2/ErbB2
3 HER3/ErbB3
4 FGFR1
5 FGFR3
6 FGFR4
7 InsR
8 IGFR-IR
9 TrkA/NTRK1
10 TrkB/NTRK2
11 Met/HGFR
12 Ron/MST1R
13 Ret
14 ALK
15 PDGFR
16 c-Kit/SCFR
17 FLT3/Flik2
18 M-CSFR/CSF-1R
19 EphA1
20 EphA2
21 EphA3
22 EphB1
23 EphB3
24 EphB4
25 Tyro3/Dtk
26 Axl
27 Tie2/TEK
28 VEGFR2/KDR
29 Akt/PKB/Rac
30 Akt/PKB/Rac
31 p44/42 MAPK (ERK1/2)
32 S6 Ribosomal Protein
33 c-Abl
34 IRS-1
35 Zap-70
36 Src
37 Lck
38 Stat1
39 Stat3
TNF-α activated STAT1 and IL6 activated STAT3

- Untreated
- TNF-α treated
- IL6 treated

HT-29
Cell lines with a low STAT1/high STAT3 ratio showed faster tumor growth in xenografts.

In contrast, xenografts of cell lines showing high STAT1 and low STAT3 levels grew slower.

Importantly, these ratios reflected clinical outcome in CRC patients as well.
STAT1/STAT3/NF-κB formed triplet complex upon cytokine stimulation, Withaferin A abolished the protein-protein interactions
Summary

• IL6 and TNF-a induced STAT3 activation that is important for stemness in colorectal cancer cells.

• Combined cytokines converted cancer cells to more stem-like cells.

• STAT3/STAT1/NF-kB formed complexes upon cytokine stimulations.

• Natural compound withaferin A has abolished protein-protein interactions and reduced telomerase activity in colorectal cells.
Hallmarks of cancer: the next generation.
Case based question

• A 64-year-old male patient underwent a right hemicolecotmy revealing a moderately differentiated adenocarcinoma, staged as a T4N0 (the tumor has grown through the outer lining of the colon; no lymph nodes containing cancer cells) colon cancer. Eight lymph nodes were removed at the time of surgery and all were negative for metastatic disease. No adjuvant chemotherapy was administered.

• Approximately two years later, the patient was found on routine evaluation to have a rising carcinoembryonic antigen. A PET/CT scan was performed revealing three small lesions within the liver. Biopsy confirmed the presence of metastatic colon cancer. The patient was started on 5-FU and oxaliplatin (Eloxatin, Sanofi Avenitis; FOLFOX) in combination with bevacizumab (Avastin, Genentech).

• He was stable on this regimen for approximately 13 months. Follow-up scans revealed new liver metastases and a single isolated pulmonary nodule. In this patient with metastatic colon cancer that has progressed after treatment with FOLFOX plus bevacizumab, the most appropriate treatment recommendation would be which of the following:

  A. Single-agent irinotecan.
  B. FOLFIRI with bevacizumab.
  C. An irinotecan-based chemotherapy in combination with cetuximab (Erbitux, Imclone).
  D. Continuation of bevacizumab as a single agent.
Discussion

• In this patient, treatment with an irinotecan-based chemotherapy regimen would be the favored approach.

• In patients who are wild-type for the KRAS mutation, the addition of cetuximab is also reasonable.

• After withaferin study: Clinical enrollment in which the addition of withaferin A is being investigated, it could be additional consideration.
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