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TITLE: Prostate Cancer Models of Genomic Instability

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Introduction
Genomic instability is a common feature of human cancer, and is associated with aberrant mitosis, aneuploidy, and other chromosomal irregularities [1]. Genomic instability likely arises from centrosome abnormalities that induce spindle defects, leading to imbalanced chromosomal segregation. In fact, centrosome anomalies arise in early prostate cancer and occur more frequently in high-grade cancers [2]. While the molecular events of genomic instability are not fully understood, their characteristics are tightly associated with aberrant expression of proteins controlling DNA damage response and cell cycle checkpoints such as the mitotic kinase Aurora-A and -B, and checkpoint protein Bub1. Overexpression of Aurora-A and -B are overexpressed in many transformed and cancer cell lines and their levels increases as a function of tumor state in many different types of cancers [3, 4]. Overexpression of Aurora-A kinase induces centrosome amplification, aneuploidy, abnormal cell cycles, and transformation of p53-deficient mammalian cells [5]. Inactivational mutation in Bub1, although rare, functions dominantly in some cancers and induces chromosomal instability and increase aneuploidy [6, 7]. In addition, recently studies have shown that inactivation of Bub1 activity is essential for SV40 large T-antigen-induced neoplastic transformation [8].

Key Research Accomplishments
- Shown that the steady-state expression levels of Aurora-A and -B, and Bub1 in human prostate cancer cell lines are positively correlates with their tumorigenecity and invasive potential.
- Shown that TRAMP cell lines express high levels of Aurora-A and -B, and low levels of Bub1.
- Shown that TRAMP mice express high level of Aurora-A and Aurora-B in the prostatic tumors while only low to moderate level (AP=DP<LP<VP) of the proteins were found in the prostatic tissue of age-matched non-transgenic mice.
- Identified a novel mis-sense mutation of the p53 gene (ΔT812) in TRAMP cell lines that may play a significant role in tumorigenesis of the prostate.

Reportable Outcomes
I) Status of Aurora-A, Aurora-B and Bub1 in Human and TRAMP tumors
We have demonstrated that the steady-state expression levels of Aurora-A, Aurora-B, and Bub1 in LNCaP, DU145 and PC3 human prostate cancer cell lines is positively correlated with their tumorigenecity and invasive potential (Figure 1). We have also demonstrated that TRAMP cell lines express high levels of Aurora-A and Aurora-B and low levels of Bub1 (Figure 2). In addition, TRAMP mice express high level of Aurora-A and Aurora-B in the prostatic tumors while only low to moderate level (AP=DP<LP<VP) of the proteins were found in the prostatic tissues of age-matched non-transgenic mice (Figure 3 and 4).

Figure 1

A

<table>
<thead>
<tr>
<th></th>
<th>Doubling time (h)</th>
<th>Tumorigenicity</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aurora A (95% C.I.)</td>
</tr>
<tr>
<td>PC3</td>
<td>50.0</td>
<td>++</td>
<td>23.43 (9.30-62.20)</td>
</tr>
<tr>
<td>DU145</td>
<td>35.0</td>
<td>++</td>
<td>8.31 (7.26-9.25)</td>
</tr>
<tr>
<td>LNCaP</td>
<td>34.0</td>
<td>+</td>
<td>4.46 (3.07-5.15)</td>
</tr>
<tr>
<td>HeLa</td>
<td>37.0</td>
<td>-</td>
<td>11.60 (9.96-13.64)</td>
</tr>
<tr>
<td>293T</td>
<td>20.0</td>
<td>-</td>
<td>2.31 (1.99-2.68)</td>
</tr>
<tr>
<td>Normal Human Prostate</td>
<td>NA</td>
<td>-</td>
<td>1.00 (0.83-1.20)</td>
</tr>
</tbody>
</table>

B

human Aurora-A

C

human Aurora-B

D

human Bub1

E

human Bub1
II) Construction of Cre-Inducible Luciferase-linked Transgenic Mice for NmBub1 and Aurora-B
We have generated a construct of pEF1α-loxP-CAT-loxP-MCS-ires-Luciferase (designated Cre-Inducible Luciferase-linked Transgenic (CILT) system) that was designed to induce expression of both the transgene and luciferase enzyme by Cre-mediated recombination. We then subcloned mouse Myc-tagged dominant-negative Bub1 (Myc-NmBub1) and V5-tagged rat Aurora-B (V5-rAuroraB) into the CILT system and generated two constructs: CILT-Myc-NmBub1 and CILT-V5-AuroraB. These constructs have been sequence-verified (data not shown). Functional analyses have confirmed their effectiveness to express both the transgene and luciferase enzyme after Cre-induced floxing of the construct (Figure 5 and 6). These two constructs have been isolated, purified, microinjected into C57BL6/CBA ES cells and transplanted into pseudo-pregnant C57BL6/CBA females. Four individual CILT-Myc-NmBub1 founder lines (designated NMG1859, NMG1865, NMG1869 and NMG1870) and three individual CILT-V5-AuroraB founder lines (designated NMG1997, NMG2000 and NMG2003) were identified. The transgene copy numbers of these founder lines as determined by qPCR are shown in Table 1. Characterizations of the transmittance and pathobiology of these founder lines are currently underway.
Table 1

<table>
<thead>
<tr>
<th>Construct</th>
<th>Founder Line</th>
<th>Transgene Copy Number (per diploid)</th>
<th>Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CILT-Myc-NmBub1</td>
<td>NMG1859</td>
<td>1.14 ± 0.09</td>
<td>To be determined</td>
</tr>
<tr>
<td></td>
<td>NMG1865</td>
<td>2.15 ± 0.30</td>
<td>To be determined</td>
</tr>
<tr>
<td></td>
<td>NMG1869</td>
<td>3.16 ± 0.10</td>
<td>To be determined</td>
</tr>
<tr>
<td></td>
<td>NMG1870</td>
<td>3.19 ± 0.96</td>
<td>To be determined</td>
</tr>
<tr>
<td>CILT-V5-rAuroraB</td>
<td>NMG1997</td>
<td>13.98 ± 1.02</td>
<td>To be determined</td>
</tr>
<tr>
<td></td>
<td>NMG2000</td>
<td>4.13 ± 1.79</td>
<td>To be determined</td>
</tr>
<tr>
<td></td>
<td>NMG2003</td>
<td>0.64 ± 0.09</td>
<td>To be determined</td>
</tr>
</tbody>
</table>
III) Status of p53 in TRAMP tumors
We have amplified, isolated and sequenced p53 cDNA from different TRAMP cell lines and identified a point deletion at nucleotide T812 that leads to a frameshift mutation at codon 272 and a premature stop at codon 341 where part of the DNA binding domain (codon 102-292) and all of the tetramerization (codon 323-356) and negative regulation (codon 363-391) domains were aberrated (Figure 7).

Figure 7
A. Mouse p53(wt)
MTAEEQGSQDSLLELPSEQFRSLWKLLEPLDPILSPHCMDLDDLPDQVEEFFEGPSEALRVSGAAQDPVTETPGPVAPAPATPW
PLSSFVPVQKYQNYFHLGFLVQSGTAKVMVSYPLNKLFCQLAKTCPVQLVVSATPPAGSRLRAMAIYKKSQHMTEVRRC
HHFRCSDGGLAPQHLRVEGLNYPELEDROTFRHSHVYPYEPAGSEYTTIHYKVMCNSSCMGMRRPILTIITLEDSSGNLL
GRDSFEVRV271CACPGRDDRTDEENNFRKKEVLCPERPASSAKRALTCTSAASPPQKKKPLDGEYFTLKRGRKRFEMFRELNEALE
LKDAHATEESGDSRAHSSYLKTKQGSTSRHKKTMVKVGPDSDE341

B. Mouse p53(ΔT812)
MTAEEQGSQDSLLELPSEQFRSLWKLLEPLDPILSPHCMDLDDLPDQVEEFFEGPSEALRVSGAAQDPVTETPGPVAPAPATPW
PLSSFVPVQKYQNYFHLGFLVQSGTAKVMVSYPLNKLFCQLAKTCPVQLVVSATPPAGSRLRAMAIYKKSQHMTEVRRC
HHFRCSDGGLAPQHLRVEGLNYPELEDROTFRHSHVYPYEPAGSEYTTIHYKVMCNSSCMGMRRPILTIITLEDSSGNLL
GRDSFEVRV271VPALGETAVQKKKISAKRKSFAFLNCPQAQRERECPAQPALPRLPRKKNLMESIIPRSASAVNASRCSGS341

IV) Construction of Cre-inducible Luciferase-linked Transgenic Mice for p53 mutants
We have subcloned HA-tagged mouse p53 (wildtype) into the CILT system and generated the CILT-HAmp53(wt) construct. Functional analyses have confirmed its effectiveness to express both the wildtype p53 protein and luciferase enzyme after Cre-induced floxing of the construct (Figure 8). CILT-constructs with different HA-tagged p53 mutants (R172H, R172L, G242S, R279Q) were then generated by site-directed mutagenesis. These constructs have been sequence-verified and their effectiveness to express both the p53 protein and luciferase enzyme has been confirmed (data not shown). These constructs will be isolated, purified, microinjected into C57BL6/CBA ES cells and transplanted into pseudo-pregnant C57BL6/CBA females for the identification and characterization of their founders.

Figure 8

V) Effects of Transgene on Aneuploidy in TRAMP C1A Mouse Prostate Cancer Cells
We have transfected TRAMP C1A mouse prostate cancer cells with the pcDNA3.1-Myc-NmBub1 construct. Upon expression of the Myc-tagged NmBub1, C1A cells have shown a significant increase in aneuploidy (Figure 9A). This increase was further exemplified after treatment with the microtubules destabilizing agent
Nocodazole (0.2 μg/mL) (Figure 9B). The possible synergistic effects of NmBub1, Aurora-B and different p53 mutants on prostate cancer cell’s aneuploidy are currently under investigation.

Figure 9

A

Myc-NmBub1

<table>
<thead>
<tr>
<th>0 days</th>
<th>5 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
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<td><img src="d" alt="cell image" /></td>
<td><img src="g" alt="cell image" /></td>
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<tr>
<td><img src="c" alt="cell image" /></td>
<td><img src="f" alt="cell image" /></td>
<td><img src="i" alt="cell image" /></td>
</tr>
</tbody>
</table>

B

Control

Myc-NmBub1

- [0 h, 0.2 μg/mL Nocodazole](#)
- [16 h, 0.2 μg/mL Nocodazole](#)
- [40 h, 0.2 μg/mL Nocodazole](#)

Summary

<table>
<thead>
<tr>
<th>Construct</th>
<th>Functional Analyses</th>
<th>Founder Lines Established?</th>
<th>Mouse Pathobiology</th>
<th>Effects on aneuploidy in TRAMP cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CILT-Myc-NmBub1</td>
<td>Completed</td>
<td>Y</td>
<td>In Progress</td>
<td>Y</td>
</tr>
<tr>
<td>CILT-V5-rAurora-B</td>
<td>Completed</td>
<td>Y</td>
<td>In Progress</td>
<td>In Progress</td>
</tr>
<tr>
<td>CILT-HA-mp53(wt)</td>
<td>Completed</td>
<td>N</td>
<td>-</td>
<td>In Progress</td>
</tr>
<tr>
<td>CILT-HA-mp53(R172H)</td>
<td>Completed</td>
<td>N</td>
<td>-</td>
<td>In Progress</td>
</tr>
<tr>
<td>CILT-HA-mp53(R172L)</td>
<td>Completed</td>
<td>N</td>
<td>-</td>
<td>In Progress</td>
</tr>
<tr>
<td>CILT-HA-mp53(G242S)</td>
<td>Completed</td>
<td>N</td>
<td>-</td>
<td>In Progress</td>
</tr>
<tr>
<td>CILT-HA-mp53(R279Q)</td>
<td>Completed</td>
<td>N</td>
<td>-</td>
<td>In Progress</td>
</tr>
</tbody>
</table>

Conclusions

Data supports our hypothesis that Aurora-A, Aurora-B and Bub1 play important roles in the initiation and progression of prostate cancer. Transgenic models are currently being established and characterized.

Selected References


Publications

Lee EC, Ruiz M., Greenberg N.M. Prostate Cancer and Genomic Instability. [Abstract of poster presented at the 11th Prostate Cancer Foundation Scientific Retreat, Lake Tahoe, Nevada, USA. October 2004]