Cancer Stem Cells

Cancer stem cell specific therapy → Tumor regression

Conventional cancer therapy → Tumor relapse
Pathways to Cancer

mesenchymal to epithelial transition: 
failed or incomplete differentiation

childhood cancer
"embryonal tumor"

maintaining “stemness”

Pathways to Cancer

acquiring “stemness”

epithelial to mesenchymal transition: 

dedifferentiation event

adult cancer
"carcinoma"
Wilms Tumor

a paradigm to study *failed* mechanisms of cellular differentiation & organogenesis
CITED1+/SIX2+ cells are self-renewing nephron progenitors

Hendry, et. al. Pediatric Nephrol 2011
Wilms tumor

Cancer stem cells (blastema): possess both self-renewal mechanisms and capacity for epithelial transition
CITED1 cytosolic

CITED1 nuclear-enriched

Lovvorn et al. Neoplasia. 2007
A: Proportion of Protein/β-actin in relation to Stage:

- Stage I, II, III, IV
- Data distinguished by SIX2 and CITED1

B: Proportion of Protein/β-actin in relation to Histology:

- FH, UH
- Data distinguished by SIX2 and CITED1

C: Proportion of Protein/β-actin in relation to Treatment Outcome:

- Failure, Non-failure
- Data distinguished by SIX2 and CITED1

D: Western Blot analysis:

- CITED1 27 kDa
- SIX2 32 kDa
- β-Actin 42 kDa
- MCF7, COS, SIX2, CITED1, β-Actin

Children’s Oncology Group (n=40)
CITED1 promotes TGFβ/BMP7 transcription (survival pathway for metanephric mesenchyme).

CITED1 overexpression blocks differentiation of cultured MM.

represses Wnt4 signaling (differentiation pathway for MM).

CITED1 is a non-DNA binding transcriptional regulator.

F/L -> Full Length CITED1

- **FLAG**
- **Smad-4 Interacting Domain (ΔSID)**
- **ΔCR2**
- **perturbs CBP/p300 binding**

CITED1 - dependent transcription

Dominant Negative

Plus ...

- Estrogen Receptor-α
- β-catenin
CITED1 increases cellular proliferation

H³ Thymidine Incorporation (n = 6)

Disruption of \textit{CITED1} alters tumorigenicity 

ΔCR2 mutation \textit{perturbs} tumorigenicity \textit{in vivo}

RNA-interference in soft agar

<table>
<thead>
<tr>
<th>SK-pRS</th>
<th>Empty vector control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-shCITED1</td>
<td>CITED1 knockdown</td>
</tr>
</tbody>
</table>

CITED1\textsuperscript{+}/SIX2\textsuperscript{+}

STEM CELL

Complex Media

DEVELOPMENTAL BIOLOGY

RIMM-18 MM Cell Line

WT Cell Line Maintaining Stem-Like Characteristics

CANCER STEM CELL?

CITED1\textsuperscript{+}/SIX2\textsuperscript{+}

Similar Media

CANCER BIOLOGY
Profiling of VUWT20 line

Now have a library of 14 candidate VUWT lines

<table>
<thead>
<tr>
<th>Standard media components</th>
<th>Fgf2</th>
<th>Tgfa</th>
<th>IGF-II</th>
<th>Hyaluronan</th>
</tr>
</thead>
</table>

**Table:**

- Six2
- CITED1
- sFRP2
- OSR1
- Pax2
- Sall1
- Eya1
- FoxD1
- FLK1
- Lim1
- E-cadherin
- CAD11
- Jagged1
- Sox11
- Oct4
- NCAM
- PECAM
- GAPDH
Cited1-LacZ
Reporter Mouse

heart

liver

liver

fl

liver

kidney
Hepatoblastoma

#1 liver
#3 abdominal tumor of childhood

- 100-150 cases / yr USA
- peak age = 6m to 3yr
- 5 yr survival 52-75%
Ontogeny of CITED1 in murine hepatic primordia
CITED1 expression in hepatoblastoma

Mixed
(embryonal rich)

Mixed

Pure fetal

Differentiation

CITED1+

CITED1-
CITED1 Expression in Pure Fetal vs. Mixed Histology Hepatoblastomas: Children’s Oncology Group, n=20

A

CITED1 RNA

Avg. Normalized Fold Change (log2 scale)

Fetal    Mixed

Histology

B

CITED1 Protein

CITED1/beta-actin

Fetal    Mixed

Histology
amino acid L158A 193

GFP

CITED1-wt

ΔNES

CITED1-ΔNES

HEK293
CITED1 mis-expression in Wilms tumor & hepatoblastoma: proliferation

WiT49

HEP293

No. of Proliferating Cells

ΔNES

CITED1-wt

pcDNA3

mis-expression

*p<0.001

Hours in Culture

1 hour 24 hours 48 hours 72 hours

Hours

0 24 48 72
Anchorage-independent growth

WiT49 cells
Human WT

NES 5.01-fold > pcDNA3
NES 2.22-fold > CITED1
CITED1 2.26-fold > pcDNA3
CITED1 and the “stem state”

Affymetrix GeneChip Array
pcDNA3-CITED1-wt v. pcDNA3-ev

#1 down regulated gene

\[ \downarrow \text{Cdh6} \]

Onset of epithelial transition

\begin{align*}
\text{d E12.5} & \\
\text{Cdh6+} & \\
\text{RV} & \\
\text{Renal vesicle} & \\
\text{e E13.5} & \\
\text{Comma- and S-shaped bodies} & \\
\text{ureteric bud} & \\
\end{align*}

#1 up regulated gene

\[ \uparrow \text{Lgr5} \]

Implicated in a variety of GI tract carcinomas as a marker of “stemness”

Intestinal Stem Cells at the Crypt Base

Oates A C Development 2011;138:601-607
CITED1 repression of Wnt pathway in WiT49 cells

pcDNA3-CITED1-wt v. pcDNA3-ev

**FRAT1**
-13.45

**DKK1**
+17.75

**SFRP1**
+40.79

Real time PCR array for Wnt pathway
Rat metanephric mesenchymes (MM) in culture

SFRP1

Plisov S et al. JASN 2005;16:1632-1644
CITED1+/SIX2+ → persistence & expansion of mesenchyme
CITED1+/SIX2+ → Wnt
CITED1-/SIX2+ → self-renewing progenitors
CITED1-/SIX2+ → Wnt
CITED1-/SIX2- → self-renewing progenitors
CITED1-/SIX2- → Wnt

Wnt/β-catenin
C1/S2
metanephric mesenchyme
mesenchymal to epithelial transition
2nd hit
transforming
Wilms tumor
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