Role and Regulation of Cdk Inhibitors in Development and Cancer

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Somatic cell cycles

- G0 Quiescence
- G1 Under-P RB
- G2/R Restriction point
- M Restriction point
- S Mitogen dependent G1 progression
- Cdk1/Cyclin A
- Cdk2/Cyclin A
- Cdk4(6)/Cyclin Ds
- Mitogen dependent G1 progression
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Negative regulation of G1 progression by Cdk inhibitory proteins

- p16^INK4a, p15^INK4b
- p18^INK4c, p19^INK4d

Cyclin D1,2,3
Cdk4,6

 RB, p107, p130

Cyclin E/A
Cdk2

The "Rb pathway" in human cancer

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Ink4a loss</th>
<th>Cyclin D-Cdk4 overexpression</th>
<th>Rb loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Cell Lung</td>
<td>15%</td>
<td>5% Cyclin D1</td>
<td>80%</td>
</tr>
<tr>
<td>Non Small Cell Lung</td>
<td>58%</td>
<td>20-30%</td>
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<tr>
<td>Pancreatic Lung</td>
<td>80%</td>
<td></td>
<td></td>
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<tr>
<td>Breast</td>
<td>31%</td>
<td>50% Cyclin D1</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>60%</td>
<td>40% Cdk4</td>
<td></td>
</tr>
<tr>
<td>T-ALL</td>
<td>75%</td>
<td></td>
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<tr>
<td>Mantle Cell Lym.</td>
<td>90%</td>
<td>90% Cyclin D1</td>
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</tbody>
</table>

Two groups of Ink4 proteins

- p16^INK4a and p15^INK4b
  - Genes respond to different stress signals, including cell senescence and organismal aging
  - The two genes are closely linked on human Ch. 9p21 and are frequently co-deleted in tumors
- Not expressed during mouse development; KO strains develop normally, but mice lacking Ink4a are tumor-prone; Loss of Ink4b exacerbates the effects of Ink4a inactivation; Ink4a is lost in gliomas

- p18^INK4c and p19^INK4d
  - Expressed in stereotypic patterns during development; KO strains exhibit focal tissue abnormalities
  - Ink4c (CDKN2C), 1p32 is a haplo-insufficient tumor suppressor gene; Deletions and mutations are rare, but epigenetic silencing is frequent in some tumor types including gliomas and medulloblastoma
  - No evidence that Ink4d, 19p13 is a tumor suppressor gene
Two groups of CIP/KIP proteins

- **p21Cip1**
  - Gene respond to different stress signals
  - Not expressed during mouse development; KO strains develop normally

- **p27Kip1, p57Kip2**
  - Expressed in stereotypic patterns during development in postmitotic cells, neurons in the CNS
  - Kip1/KO strain exhibit organomegaly, pituitary tumors late in life
  - Kip2 loss induces many developmental anomalies: Beckwith/Wiedemann syndrome
  - Kip1 is a haplo-insufficient tumor suppressor gene; Deletion is rare, but epigenetic silencing is frequent in some tumor types including gliomas and medulloblastoma

Expression of INK4 genes during embryogenesis

Expression of Ink4 genes in tissues of young mice

Role of p18Ink4c and p19Ink4d in spermatogenesis
Testicular atrophy but fertility in Ink4d-null male mice

Testicular atrophy is associated with increased apoptosis

A. LacZ staining

\[\begin{array}{c}
+/- \\
-/- \\
\end{array}\]

B. Tunel assay

\[\begin{array}{c}
+/- \\
-/- \\
\end{array}\]

Ink4c/Ink4d double deficient male mice are sterile: absence of spermatozoa due to delayed meiosis I and increased apoptosis

Conclusions

- p18Ink4c and p19Ink4d are essential for male fertility
- Both Ink4 proteins collaborate to ensure mitotic exit and normal meiotic maturation of spermatocytes
Role of Cdk inhibitors in the CNS

Hypothesis

- Neurons must exit cell cycle to differentiate and migrate to their right positions in the brain.
- Loss of cell cycle inhibitory proteins that enforce Rb tumor suppressor functions may lead to cancers in the CNS.

Role of Cdk inhibitors in the CNS (2)

- In the developing CNS, p18\textsuperscript{Ink4c} is expressed transiently in proliferating neurons and time their exit from the cell cycle while p19\textsuperscript{Ink4d} and p27\textsuperscript{kip1} are found mainly in post-mitotic neurons.
- p19\textsuperscript{Ink4d} and p27\textsuperscript{kip1} expression is maintained in adult brain and actively keep neurons out of cycle.
- p27\textsuperscript{kip1}-null mice are deaf due to increased proliferation of supporting cells in the organ of Corti.
Progressive sensory hair cell loss in Ink4d-/- mice induces progressive deafness

Ink4d is required for the active maintenance of the post-mitotic state in sensory hair cells

Postnatal development in Ink4d, Kip1-null mice

Mice die by 18 days after birth with major neurological defects
Conclusions (2)

- p19\textsuperscript{Ink4d} and p27\textsuperscript{Kip1} are required to actively keep differentiated neurons out of cycle
- Loss of either protein induces deafness, loss of both induces neurological defects and death by day 18 after birth, but no brain tumors

Role of the Rb pathway in cerebellar development

- Cyclins D2 and D1 by binding to Cdk4 regulate cerebellar development (Sicinski's lab)
- \textit{N-Myc}-dependent suppression of p18\textsuperscript{Ink4c} and p27\textsuperscript{Kip1} expression is required for proper cerebellar development (Knoepfler, Eisenman)
- Deletion of Rb and p53 in the cerebellum induces 100% medulloblastoma (Marino, Berns)

Medulloblastoma

- Represents the most common pediatric brain tumor
- Cerebellar neuronal tumor, peak incidence ages 2-7 years
- Arises from granule neuron precursors (GNPs) that proliferate and differentiate in the postnatal cerebellum
- ~25% of human primary tumors have mutational defects in sonic hedgehog or Wnt signaling pathways
- ~10% have p53 mutations
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Childhood medulloblastoma

Average Risk
- ~75% long term survival rate

Surgery
Chemotherapy
Radiotherapy

High risk
- ~50% long term survival rate

Better therapies are required to improve survivors' quality of life

Cerebellum = little brain

- Represents only 10% of the total brain mass, hence its Latin name, but contains >50% of our neurons
- It coordinates sensory inputs from the periphery to fine tune our movements and balance
- Although it is the first brain structure to differentiate, its maturation occurs mainly after birth for 16 months in humans, two weeks in the mouse
- This protracted developmental process makes the cerebellum an easier brain structure to study, but it also makes it especially vulnerable to developmental anomalies, leading to structural defects and cancer

Postnatal cerebellar development

External Granular Layer (EGL)
Internal Granular Layer (IGL)
Molecular Layer (ML)

Adapted from Cynthia Wetmore
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Cerebellar development in the mouse

The (simplified) SHH/PATCHED signaling pathway

Rb-dependent cell cycle exit and differentiation of GNPs

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Ink4c expression in the mouse cerebellum

P27Kip1 is expressed in non-dividing granule cells and its expression is maintained in the IGL throughout adulthood.

N-Myc in GNP proliferation

Wei Du, Sicinski, McKinney & Rawlitch, Knoepfler & Eisenman
Partial rescue in adult cerebellum

WT  Ink4c/Kip1/N-mycF/F  Nestin-Cre+
N-mycF/F  Nestin-Cre+

In coll. with Knoepfler and Eisenman

Hypothesis (2)

By canceling the growth suppressive functions of Rb-family proteins, loss of Ink4c, Kip1, alone or in combination, may contribute to tumor formation by providing cerebellar granule neuronal precursors (GNPs) with a proliferative advantage.

Results

Mice lacking Kip1, Ink4c, or both exhibit organomegaly, which includes the cerebellum, but there are no overt defects in cerebellar or CNS functions.

Mice lacking Ink4c and p53 develop medulloblastoma (25%) with anaplastic features at 2-5 months of age.

Crosses into an Arf-null background did not induce tumors.
Ink4c loss increases medulloblastoma incidence in Ptch1 +/- mice

The wild-type Ptc allele is lost in MBs, but there is no LOH at the Ink4c locus; therefore, p18 is haplo-insufficient for tumor suppression.

Do p18^{INK4C} contribute to human medulloblastoma?

- No loss of copy number of INK4c was detected by array CGH in 46 primary medulloblastomas (SJCRH).
- 4/23 (17%) human medulloblastomas exhibited partial (3 cases) or complete (1 case) methylation of the CDKN2C/INK4C promoter.
- No normal human cerebellar tissues (0/9) exhibited CDKN2C/INK4C methylation.
- INK4a promoter was not methylated in 23/23 cases of medulloblastoma.

p18^{INK4C}/CDKN2C protein expression is lost in primary medulloblastoma

17/73 (23%)
42/73 (57%)
14/73 (20%)

Conclusions (3)

- Loss of Ink4c collaborates with the lack of p53 or patched to induce medulloblastoma in mice; Loss of Ink4c is found in ~ 20% of human medulloblastomas
- Loss of Ink4a/Arf and Ink4c (Solomon et al., April 2008; Wiedemeyer et al., April 2008) or Cdk4 overexpression induces glioblastoma when combined with mutations of EGFR or p53 in mouse and man

Phenotype of Ink4d – or Ink4c – deficient mice

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<tr>
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<th>Ink4d KO</th>
<th>Ink4c KO</th>
<th>Ink4c KO</th>
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<tbody>
<tr>
<td>Testicular atrophy</td>
<td>Organomegaly (spine, kidney, heart, adrenal gland, thymus and testis)</td>
<td>Testicular tumors (semimembranos)</td>
<td>Fertile</td>
</tr>
<tr>
<td>Fertile</td>
<td>Deafness</td>
<td>Pituitary adenoma (intermediate lobe)</td>
<td>Pituitary adenoma (intermediate lobe)</td>
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<tr>
<td></td>
<td>T-cells hyperplasia</td>
<td>Adrenal gland tumor</td>
<td>Adrenal gland tumor (pheochromocytoma)</td>
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<tr>
<td></td>
<td>(in vitro)</td>
<td>Fertile</td>
<td>Fertile</td>
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<td></td>
<td>Glomerulopathies</td>
<td>Atrophyc glomeruli</td>
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<td>Glomerular cyste</td>
<td>Glomerular cyste</td>
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Acknowledgments

Charles J. Sherr

Olivier Ayrault | Brain Tumor Program
Tamar Uziel | Steven Clifford and Sarah Millner, Univ. New Castle, UK
Haotian Zhao | Charles Eberhart, Philip A.Beachy, Johns Hopkins, Baltimore, Md
Frederique Zindy | Lee Rubin, Curis Inc., Boston
Suqing Xie | The Children's Brain Tumor Foundation (CBTF), The Pediatric Brain Tumor Foundation (PBTF), The American Brain Tumor Association (ABTA), NCI, SJCRH and ALSAC
Antoine Forget

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