Intracranial Neoplasia in Rodents

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Part I – Modeling Intracranial Neoplasia in Rodents

Part II – Spontaneous Neoplasia in Rodents
Part I – Modeling CNS Neoplasia

• Why use animal models to study CNS neoplasia?
  1. Identify the fundamental genetic and molecular mechanisms of CNS carcinogenesis, including angiogenesis, metastasis, autonomous growth and immune evasion
     • *in vivo* models are more relevant for studying many aspects of carcinogenesis
  2. To test potential therapeutics

• Rodents have historically been the most widely used *in vivo* models
  – Mammals
  – Small size (ease of manipulation and housing)
  – Relatively short life span
  – Genetic tractability, especially of mice
Types of Rodent Models

1. Syngenic models
   • Implantation of cultured cells derived from neoplasms from the same rodent strain
   • Can use immunocompetent rodents

2. Xenograft models
   • Implantation of cultured cells derived from human neoplasms
   • Must use immunodeficient rodents

3. Transgenic models
   • Genetically Engineered Mouse Models (GEMs)
Types of Rodent Models

– Syngeneic models in rats
  • 9L, C6, T9, F98, RG2, BT4C, RT-2 and CNS-1 rat glioma cell lines
    – Most derived from rats after \textit{in utero} exposure to a chemical mutagen, typically a nitrosourea compound
    – Most cell lines originally developed prior to the 1970’s
    – Injected intra-cerebrally into immunocompetent hosts
    – Historically used quite widely but less so today
      » Lack histopathologic and molecular similarity to human neoplasms
      » Still valuable in studying basic biological processes or testing therapeutics, particularly those that manipulate the tumor interface with normal tissue, e.g., angiogenesis or the immune response
Types of Rodent Models

– Syngeneic models in mice
  • P560, CT-2A, GL261, GL26, 4C8 glioma cell lines, among others
    – P560 – Spontaneous
    – CT-2A, GL261, GL26 – chemically induced with methylcholanthracene
    – 4C8 – derived from MOCH-1 glioma cell line (transgenic)
    – Historically not as widely used as rat cell lines
      » Mice are more resistant to chemical mutagenesis
    – Injected intra-cerebrally into immunocompetent hosts
    – Some cell lines, e.g., GL261, are still commonly used to test potential therapies, particularly immunotherapies
Types of Rodent Models

– Xenograft models
  • Intra-cerebral injection of neoplastic cells of human biopsy origin
    – Heterotopic (typically subcutaneous) vs orthotopic (brain)
    – Requires the use of immunodeficient rodents
    – Most commonly used for gliomas
      » Monolayer cell lines (clonal)
        » Use has limitations but still widely used for testing of therapeutics
      » Cells grown in ‘neurobasal medium’ (enriches for neoplastic cells with stem cell properties)
        » Can recapitulate a more complete histologic and molecular phenotype
        » Phenotype is more stable over time
      » Biopsy spheroids
        » Larger biopsy specimens, with support tissue and cells, grown in agar then implanted
Types of Rodent Models

- Genetically Engineered Mouse Models (GEMs)
  - Introduction of key genetic alterations identified in human neoplasms into mice
  - Neoplasms better reflect the biology of their human counterparts, including histopathology and genetics
  - Genetic modifications can be tissue specific, developmentally regulated, and multiple, particularly using new gene editing strategies such as CRISPR
  - Unlike implantation models, GEM allow for studies of tumor initiation and, since they are in immunocompetent animals, the tumor’s interaction with the immune system
Spontaneous Neoplasia in Rodents
Spontaneous Neoplasia

• Rodents are resistant to the spontaneous development of intracranial neoplasia
• Generally occurs in aged animals, as in other species
• Malignancy determination is based strictly on morphology. Terminal or premature sacrifice animals are one time-point only, so the true nature of tumors can’t always be clearly defined.
• Certain lesions that have well-differentiated features but are biologically aggressive over time (e.g., glial neoplasms) are diagnosed as "malignant, low grade" rather than “benign” to better capture their typical biological behavior.
Spontaneous Neoplasia

- Neurons
  - Primitive Neuroectodermal Tumors (PNETs)
    - Medulloblastoma
    - Olfactory Neuroblastoma
    - Neuromyoblastoma
  - Neurocytoma

- Glia
  - Astrocytoma
  - Oligodendroglioma
  - Mixed Glioma
  - Glioma
  - Pituicytoma

- Choroid plexus
  - Papilloma
  - Carcinoma

- Ependyma
  - Ependymoma

- Meninges
  - Meningioma
    - Granular cell tumour
    - Meningioma
  - Malignant Meningioma

- Schwann cells
  - Schwannoma

N.B. Pituitary and Pineal Neoplasms are not included
Historical Control Data* for Wistar-Han rats and B6C3F1 mice from the National Toxicology Program

<table>
<thead>
<tr>
<th>WISTAR/HAN RATS – Brain</th>
<th>Incidence (M)</th>
<th>Incidence (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLIOMA, high grade</td>
<td>2/299</td>
<td>0/300</td>
</tr>
<tr>
<td>GRANULAR CELL TUMOR, Benign</td>
<td>5/299</td>
<td>3/300</td>
</tr>
<tr>
<td>GRANULAR CELL TUMOR, Malignant</td>
<td>0/299</td>
<td>1/300</td>
</tr>
<tr>
<td>MENINGIOMA, Malignant</td>
<td>1/299</td>
<td>0/300</td>
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<table>
<thead>
<tr>
<th>B6C3F1 MICE – Brain</th>
<th>Incidence (M)</th>
<th>Incidence (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRANULAR CELL TUMOR, Benign</td>
<td>1/700</td>
<td>0/699</td>
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<tr>
<td>MENINGIOMA, Malignant</td>
<td>1/700</td>
<td>0/699</td>
</tr>
<tr>
<td>OLIGODENDROGLIOMA, high grade</td>
<td>1/700</td>
<td>0/699</td>
</tr>
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Historical Control Data* for CD IGS rats and CD-1 mice from Charles River Laboratories

<table>
<thead>
<tr>
<th>CD RATS – Brain</th>
<th>Incidence (M)</th>
<th>Incidence (F)</th>
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<tbody>
<tr>
<td>ASTROCYTOMA, High grade (aka. Malignant)</td>
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<tr>
<td>MENINGIOMA</td>
<td>2/1205</td>
<td>1/1205</td>
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<tr>
<td>MEDULLOBLASTOMA</td>
<td>0/1205</td>
<td>1/1205</td>
</tr>
<tr>
<td>OLIGODENDROGLIOMA, High Grade</td>
<td>8/1205</td>
<td>0/1205</td>
</tr>
<tr>
<td>SCHWANNOMA, Malignant</td>
<td>1/1205</td>
<td>0/1205</td>
</tr>
<tr>
<td>GRANULAR CELL TUMOUR, Benign</td>
<td>0/1205</td>
<td>1/1205</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD-1 MICE – Brain</th>
<th>Incidence (M)</th>
<th>Incidence (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MENINGIOMA</td>
<td>1/900</td>
<td>0/900</td>
</tr>
</tbody>
</table>

## Historical Control Data* for various mouse strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>VM (Frazier 1971)</th>
<th>BALB/c (Morgan et al. 1984)</th>
<th>B6C3F1 (NTP 2004b)</th>
<th>CD1 (Harlan 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEX</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td># OF ANIMALS</td>
<td>10,000</td>
<td>7,427</td>
<td>1,557</td>
<td>500</td>
</tr>
<tr>
<td>PREVALENCE (%)</td>
<td>0.2</td>
<td>0.03</td>
<td>0.19</td>
<td>0</td>
</tr>
</tbody>
</table>

Classification of Neoplasms

• Neuronal Neoplasms
  • Primitive Neuroectodermal Tumors (PNETs)
    • Medulloblastoma
    • Olfactory Neuroblastoma
    • Neuromyoblastoma
  • Neurocytoma
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Medulloblastoma

- **Biological behavior:** Malignant neoplasm
- **Synonyms:** primitive neuroectodermal tumor (PNET) of cerebellum
- **Histogenesis:** derived from cells of cerebellum; precise histogenesis is unknown
- **Special diagnostic techniques:** None in rat and mouse. Synaptophysin, Neuron Specific Enolase (NSE) and Neu N may be useful in non-human primates and dogs
Medulloblastoma

- **Diagnostic features:**
- Highly cellular: undifferentiated, hyperchromatic round/elongated nuclei with scant cytoplasm forming sheets and occasional rosettes; indistinct cell borders
- May see neuronal cell bodies, glial cells and even muscle fibres
- Similar neoplasm occurs in olfactory bulb (olfactory neuroblastoma)
- Bizarre mitotic figures; invasive and often replaces cerebellar folia
- **Unusual features:** tend to occur in aged rodents; specific morphologic subtypes are not recognized.
Medulloblastoma

Photo courtesy of the NTP
Medulloblastoma

Photo courtesy of the NTP
Medulloblastoma

Photo courtesy of the NTP
Olfactory Bulb Neuroblastoma

Photo courtesy of the NTP
Olfactory Bulb Neuroblastoma

Photo courtesy of the NTP
Classification of Neoplasms

- Glial Neoplasms
  - Astrocytoma
  - Oligodendroglioma
  - Glioma
  - Mixed Glioma
  - Pituicytoma
Classification of Neoplasms

• Glial Neoplasms
  – Astrocytoma
  – Oligodendroglioma
  – Glioma
  – Mixed Glioma
  – Pituicytoma
Astrocytoma

• **Biological behavior:** malignant neoplasm, varies from minimally to highly aggressive neoplasm
• **Histogenesis:** resident astrocytes?
• **Diagnostic features:** Uniform to anaplastic cellular features: round or fusiform nuclei, variable eosinophilic cytoplasm, indistinct cell borders
  – Rats - cellular differentiation into subtypes is reported:
    • **Protoplasmic:** stellate cells with delicate cytoplasmic processes that form a mesh-like matrix
    • **Fibrillary:** round cells with elongate nuclei
    • **Gemistocytic and Pilocytic (pilloid)** are less common
• Mouse astrocytomas similar to the rat protoplasmic variant
Astrocytoma

• Unusual Features:
  – Lack glial fibrillary acidic protein (GFAP) and Phosphotungstic acid haematoxylin (PTAH) reactivity
  – Typically positive for microglial markers, including Iba-1, CD68, Ox-6 (MHC II), lysozyme, vimentin
  – Contain bi-nucleate neoplastic cells with eosinophilic granules
Astrocytoma
Astrocytoma: H&E, protoplasmic morphology
Astrocytoma: fibrillary morphology
Astrocytoma: neuronal satellitosis
Astrocytoma: extension into meninges
Astrocytoma: necrosis and pseudopalisading
Oligodendroglioma

- **Biological behavior**: minimally to highly aggressive neoplasm
- **Synonym(s)**: glioma
- **Histogenesis**: oligodendrocytes
- Uncommon neoplasms, usually peri-ventricular (thalamus, basal ganglia, corpus callosum), hypothalamus
- **Diagnostic features**: variably well circumscribed; sheets, rows or nests of small cells with round, central, hyperchromatic nuclei and clear cytoplasm (‘perinuclear halo’); variable degree of anaplasia depending on grade
- Cytoplasm and surrounding stroma accumulates acid mucopolysaccharides that stain with Alcian blue
Oligodendroglioma

• **Special techniques**: Positive for MBP (myelin basic protein) galactose cerebroside, carbonic anhydrase C and Oligodendrocyte transcription factor-2 (Olig-2).
• Rare in mice unless induced, but may occur spontaneously in BALB/c.
• **Unusual features**:
  • Commonly display a prominent vascular proliferation, referred to as ‘glomeruloid vessels’ or ‘vascular garlands’
Oligodendroglioma

Photo courtesy of the NTP
Oligodendroglioma

Photo courtesy of the NTP
Oligodendroglioma

Photo courtesy of the NTP
Oligodendroglioma

Photo courtesy of the NTP
Oligodendroglioma

Photos courtesy of the NTP
Glioma

- Diagnostic term that can be used for any glial neoplasm
- Most often used if the histogenesis of the glial neoplasm is unclear using just an H&E stain
  - In toxicologic pathology, immunohistochemical stains are often not done on individual neoplasms
- Also used by several groups for what has historically been diagnosed as an ‘astrocytoma’, given the recent data on the histogenesis of that neoplasm
Meningeal Neoplasms

- Meninges
  - Meningioma
  - Malignant Meningioma
Meningioma

- *Biological behavior*: benign or malignant neoplasm
- *Histogenesis*: stromal cells of the meninges (meningeal arachnoid cell)
- *Diagnostic features*: Uncommon in rats (except for GCT, which are fairly common in certain strains); rare in mice
- Usually dorsal/dorsolateral over the cerebellum or cerebral hemispheres, but can occur in any location
- Varying degrees of compression and invasion of the underlying parenchyma
- Mitotic figures are variable depending on malignancy
- Several morphological variants occur
Granular Cell Tumor

- **Biological behavior**: benign or malignant neoplasm
- **Synonym(s)**: none
- **Histogenesis**: stromal cells of the meninges (meningeal arachnoid cell)
- **Diagnostic features**: solid, round to plaque-like, micronodular proliferations of granulated cells with fine vascular stroma
  - Benign neoplasms are most common and compress the adjacent brain
  - Malignant GCTs are rare and are invasive
- **Special diagnostic techniques**: PAS for granular cytoplasmic contents.
Granular Cell Tumor
Granular Cell Tumor

Photo courtesy of the NTP
Granular Cell Tumor

Photo courtesy of the NTP
Meningioma, fibrous

- Elongated (spindle or fusiform) cells with pale eosinophilic cytoplasm
- Nuclei are small, elongated, and have a reticular or hyperchromatic chromatin pattern
- The cells form interwoven bundles in loose or fascicular patterns, with varying amounts of collagen separating individual cells
- ‘Myxoid’ variant is most common type in mice
  - Alcian blue +ve
Meningioma, fibrous

Photo courtesy of the NTP
Meningioma, meningothelial

- Meningothelial type: large epithelioid cells with homogenous eosinophilic cytoplasm and a vesicular nucleus, arranged in lobules separated by a fibrovascular stroma.

Photo courtesy of the NTP
Meningioma, Psammomatous

- Psammoma bodies (intra-neoplastic laminar calcified concretions) are an uncommon feature of rodent meningiomas.

Photos courtesy of the NTP
Meningioma, malignant

- **Biological behavior**: malignant neoplasm.
- **Synonyms**: meningeal sarcoma.
- **Diagnostic features**: Invasive growth into the underlying brain parenchyma, typically with extensive infiltration along the adventitia of small radiating blood vessels.
- Cellular atypia, pleomorphism, high mitotic index, giant cells.
- Can display fibrosarcomatous/myxoid, meningothelial or anaplastic patterns
Meningioma, malignant

Photo courtesy of the NTP
Meningioma, malignant

Photo courtesy of the NTP
Meningioma, malignant

Photo courtesy of the NTP
Meningeal Neoplasms

- *Unusual features:*
- Limited morphologic variability compared to other species, particularly in mice
- Rodent (non-granular cell) meningiomas have a much greater tendency for malignancy than other species
  - Very highly infiltrative neoplasms in mice
  - In mice, often extend circumferentially around the brain
Acknowledgements

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• Alys Bradley, Charles River

We are committed to the humane care of the research animals produced and used in all Charles River activities.