New Insights into Merkel Cell Carcinoma

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Merkel cell carcinoma

• Clinical features:
  – Sun-exposed head, neck and upper extremities
  – Elderly patients (mean age 75), male predominance
  – Rarely in children
  – Red color often resembles angiosarcoma, but usually indistinguishable from other cutaneous neoplasms
  – Usually about 2 cm in diameter at time of presentation
Merkel cell carcinoma
Merkel cell carcinoma

- Highly aggressive neoplasm
- Incidence of 0.23/100,000 in Caucasian Americans, very rare in African Americans
- 1500 new cases/year in USA – incidence rising rapidly
- Local recurrence 25%
- Metastasis to regional nodes 50%
- Distant metastases 34%
- Death 34%
Merkel cell carcinoma

- **Histologic features:**
  - Small round, uniform cells distributed in sheets and trabeculae
  - Vesicular nucleus, inconspicuous nucleoli
  - “salt-and-pepper” chromatin
  - Minimal cytoplasm
  - Multiple mitoses and apoptotic cells
  - Epidermotropism in about 10% of cases
  - Often areas with divergent differentiation (SCC, BCC, rarely melanocytic)
Merkel cell carcinoma
Merkel cell carcinoma
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Merkel cell carcinoma

- Immunohistochemical features:
  - Cytokeratin positive (dot-like pattern – paranuclear)
  - CK20 sensitive marker (not totally specific)
  - TTF-1 positive in small cell carcinomas of lung, but also rarely positive in MCC
  - Somatostatinin and chromogranin frequently positive
  - NSE and EMA also positive but very non-specific
  - S100 negative
Merkel cell carcinoma

CK20
Merkel cell carcinoma

CK20
Merkel cell carcinoma

Chromogranin
Merkel cell carcinoma

- Indicators of poor prognosis:
  - Male
  - Age > 55 years
  - Location on head and neck
  - Advanced stage at time of diagnosis
  - Tumor > 2 cm
  - Immunosuppression
  - Diffuse growth pattern
  - Heavy lymphocytic infiltrate
  - High mitotic rate
  - P63 expression
Merkel cell carcinoma: staging and prognosis

• 5 year survival rates:
  – Stage I: (T1 N0 M0 - primary tumor < 2 cm) – 81%
  – Stage II: (T2 N0 M0) – primary tumor ≥ 2 cm) – 67%
  – Stage III: (any T, N1 M1) – 52%
  – Stage IV: (any T, any N M1) – 11%

Insights into Merkel cell carcinoma

• Etiology and Pathogenesis
• Prognosis
  – Histologic
  – Immunohistochemical
  – Cytogenetic
• Therapy and treatment
Merkel cell carcinoma

• Insights into etiology
  – Probably NOT derived from cutaneous “Merkel” cells of the skin, but rather, likely originates from pluripotential stem cells that undergo neuroendocrine differentiation
  – Many articles citing:
    • Merkel cell carcinoma + SCC (J Cutan Pathol 2008; 35: 955-959)
    • Merkel cell carcinoma + melanocytic tumor
    • Merkel cell carcinoma + fibrosarcomatous differentiation (Pathology 2008; 40:314)
Merkel cell carcinoma

• Insights into pathogenesis
  – Strong association with presence of MC-polyomavirus
    • Virus found in integrated and clonal form in 70% of MCC (only 10 cases)
    • Polyomavirus has proven transforming abilities in mammalian cells – related to SV40 virus
    • Some cases were clearly negative, so not “necessary” for the development of MCC

Merkel cell carcinoma

- Immunohistochemical advances in diagnosis:
  - Achaete-scute complex-like I (MASH1, ASCL1) involved in development of brain and neuroendocrine system
  - 30 MCC compared with 59 small cell carcinoma of lung with anti-MASH1 antibodies
  - 83% of small cell carcinoma expressed MASH1 and 73% expressed TTF-1
  - 0% of MCC expressed MASH1 while 1/30 (3%) expressed TTF-1
  - MASH1 may be superior to TTF-1 in making this distinction

Histologic contributions to prognosis

- High mitotic rate
- Depth of invasion*
- Small cell size*
- Angiolymphood invasion*
- Diffuse growth pattern*
- Heavy lymphocytic infiltrate”

*Not all studies agree
Histologic contributions to prognosis

• Depth of invasion:
  – no relationship between tumor thickness and disease free survival or overall survival

  – MCC >10 mm thick associated with higher rate of distant metastases

  Llombart B et al. Histopathology 2005; 10.111:1
Histologic contributions to prognosis

• Depth of invasion:
  – Extension of tumor into the subcutaneous fat was strongly predictive of shortened survival in study of 25 patients

Mott RT et al. J Cutan Pathol 2004; 31: 217
Histologic contributions to prognosis

• 36 MCC evaluated for numbers of mast cells as identified by mast cell tryptase
• Significant relationship between tumor mast cell count and survival
• 1.75 increase in risk of death for each additional mast cell per 250X field

**Immunohistochemical contributions to prognosis**

- **Survivin expression**
  - 16.5 kDa intracellular protein – functions as inhibitor of apoptosis – prevents activation of caspases
  - Expressed in 19/19 cases (Yale Univ.)
  - Nuclear staining pattern associated with high rate of metastasis (50%) and mortality (38%) within 3 year f/u period
  - Cytoplasmic staining associated with increased disease free survival

Survivin expression in Merkel cell carcinoma

Nuclear staining

Cytoplasmic staining
Immunohistochemical contribution to prognosis

• P63 expression
  – 47 cases of MCC stained with p63 antibodies
  – 25/47 demonstrated staining with p63
  – More aggressive course (lower overall survival) in tumors with p63 expression (P = .0003)
  – Caveat – p63 expression correlated with Ki-67 staining, so may not be an independent predictor

P63 Expression in Merkel cell carcinoma
Immunohistochemical contributions to prognosis

• Tissue microarray of 31 MCC (15 free of disease, 16 metastasized)
• 43 markers examined
• Over expression of following associated with metastasis:
  – Matrix metalloproteinase (MMP) 7
  – MMP 10/2
  – Tissue inhibitor of metalloproteinase 3
  – Vascular endothelial growth factor (VEGF)
  – P38
  – Stromal NF-kappa B
  – Synaptophysin

Fernandez-Figueras et al. Mod Pathol 2007; 20: 90
Cytogenetics and Merkel cell carcinoma

- Trisomy 6 present in > 60% of cases of MCC, but not all
Current treatments for Merkel cell carcinoma

• Wide local excision – standard therapy
  – < 1 cm margins NOT associated with higher risk of recurrence
  – 2 cm margins best reserved for lesions > 2 cm

2008
Current treatment options for Merkel cell carcinoma

• Sentinel node (SN) biopsy – controversial
  – 241 patients from about 20 studies in literature suggest that about 30% of patients have positive SNs at time of presentation
  – 18.7% recurrence rate for patients with positive SN vs. 7.5% recurrence for those with negative SN

Current treatment options for Merkel cell carcinoma

• Adjuvant post-operative radiation therapy – also controversial
  – SEER registry:
    • Improved median survival for RT + surgery over surgery alone for primary tumors of all sizes
  – MSKCC – adjuvant RT added nothing to surgery alone for survival (only a small percentage of patients received RT in this series)
  – No difference in 3 year survival between patients who received adjuvant RT or chemotherapy and those who didn’t (Gupta et al. Arch Dermatol 2008; 142: 685.)
Current treatment options for Merkel cell carcinoma

• Adjuvant chemotherapy:
  – Of little use at this time – most Merkel cell carcinomas do not respond to standard chemotherapeutic regimens
  – Current work addresses targeting therapies directed against antigens expressed by neoplastic Merkel cells
Histologic contributions to treatment options

• 32 MCC analyzed using tissue microarray for expression of following antigens:
  – C-kit (CD117)
  – Vascular endothelial growth facts A, C, VEGF receptor-2
  – Platelet-derived growth factors α and β
  – Epidermal growth factor receptor
  – Her-2/Neu
  – Mcl-1, Bmi-1

• All except c-kit, Her-2/Neu and EGFR uniformly expressed by vast majority of MCC by immunohistochemistry
Contributions to treatment options in MCC

- Her-2/Neu – involved in protein kinase signaling network – ligand binding domain – absence in MCC suggests this is not an effective potential therapeutic option
- Receptor tyrosine kinases (VEGF, PDGF) – expressed in low levels in this study (perhaps more expression in larger cell type)
- C-kit – no mutations found so not very promising treatment option
- Anti-sense Mcl-1 and Bmi-1 oligonucleotides suggest possible promise

Mcl1 and Bmi-1

- Genes are involved in cell proliferation and cell death
  - MCI-1 is a member of the bcl-2 family – anti-apoptotic – promotes cell survival
  - Bmi-1 is a transcriptional repressor – over expression may be related to immortality in cancer cells
- Introduction of antisense oligonucleotides targeting these genes may reverse their roles
- Anti-sense Bmi-1 oligonucleotide inhibits proliferation in some leukemias
- Neither yet tried in Merkel cell carcinomas
Contributions to treatment options for MCC

- Platelet growth factor receptor α mutation found in exon 10 in 3/9 MCC – immunohistochemistry results and PCR results were identical
- PDGF-α is a transmembrane receptor tyrosine kinase
- Transmits extracellular signals into cells that activate and control proliferation, differentiation, survival and apoptosis
- If truly mutational, imatib mesylate may be useful as a treatment option

Merkel cell carcinoma – theories of oncogenesis

<table>
<thead>
<tr>
<th>Cancer-associated pathway/gene</th>
<th>Likely relevant</th>
<th>Summary of findings</th>
<th>Reference</th>
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<tbody>
<tr>
<td>p53</td>
<td>–</td>
<td>No mutations found in 12 of 15 samples</td>
<td>Van Gele et al., 2000</td>
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<tr>
<td>Ras</td>
<td>–</td>
<td>No activating mutations in H-ras, K-ras, or N-ras found in six MCC cell lines</td>
<td>Popp et al., 2002</td>
</tr>
<tr>
<td>B-RafV600E</td>
<td>–</td>
<td>No mutations in 46 MCCs</td>
<td>Houben et al., 2006</td>
</tr>
<tr>
<td>MAP kinase activity</td>
<td>–</td>
<td>MAP kinase silenced in 42/44 MCCs</td>
<td>Houben et al., 2006</td>
</tr>
<tr>
<td>Wnt</td>
<td>–</td>
<td>No mutations in β-catenin, APC, AXIN1, or AXIN2 in 12 MCC tumors</td>
<td>Liu et al., 2007</td>
</tr>
<tr>
<td>c-Kit</td>
<td>–</td>
<td>No activating mutations in nine MCC tumors</td>
<td>Swick et al., 2007</td>
</tr>
<tr>
<td>PTEN</td>
<td>?</td>
<td>No mutations in 20 of 21 samples but loss of heterozygosity for region in 43%</td>
<td>Van Gele et al., 2001</td>
</tr>
<tr>
<td>bcl-2</td>
<td>+</td>
<td>High expression in 15 of 20 MCC tumors; bcl-2 antisense decreases tumor size in xenograft model</td>
<td>Kennedy et al., 1996; Plettenberg et al., 1996; Schlagbauer-Wadl et al., 2000</td>
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