Advantages and Limitations of Assessing Proliferation in Adrenal Tumors for Diagnosis and Prognosis

Thomas Giordano

Disclosure of Relevant Financial Relationships

USCAP requires that all planners (Education Committee) in a position to influence or control the content of CME disclose any relevant financial relationship WITH COMMERCIAL INTERESTS which they or their spouse/partner have, or have had, within the past 12 months, which relates to the content of this educational activity and creates a conflict of interest.

Relevant Financial Relationship(s)
None

Overarching Statement

“Adrenal Cortical Carcinoma is an especially Proliferation-driven Neoplasm”

Role of Proliferation – Mitotic Rate

- Assessment of proliferation via mitotic rate is a component of existing diagnostic and prognostic schemes
  - Hough et al. (1979)
  - Weiss (1984)
  - van Slooten et al. (1985)
  - Weiss et al. (1989)
  - Aubert et al. (2002)
  - Pemanen et al. (2015) “Helsinki score”
Role of Proliferation - Molecular and Genomic Studies

- Dominant role of cell proliferation confirmed by numerous molecular and genomic studies

Functional Enrichment Analysis of the ACC Gene Set

- Analyzed the 2875 genes to determine if the specific genes on the list are overrepresented in other biologically relevant lists
- Compared our list to several other biologically-relevant lists
  - Gene Ontology
  - KEGG pathways terms
  - Molecular Signatures Database
  - microRNA target gene lists
  - list of genes assigned to specific chromosome arms

<table>
<thead>
<tr>
<th>Group of gene lists</th>
<th>Title of set</th>
<th># of genes on list</th>
<th># genes selected as up in ACC</th>
<th>P-value, one-sided</th>
<th>Bonferroni corrected P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEGG</td>
<td>Cell cycle</td>
<td>111</td>
<td>42</td>
<td>2.95E-28</td>
<td>5.40E-26</td>
</tr>
<tr>
<td>GO biological process</td>
<td>Cell cycle</td>
<td>376</td>
<td>80</td>
<td>2.25E-32</td>
<td>9.71E-30</td>
</tr>
<tr>
<td>GO biological process</td>
<td>Mitosis</td>
<td>123</td>
<td>47</td>
<td>1.04E-31</td>
<td>4.51E-29</td>
</tr>
<tr>
<td>GO biological process</td>
<td>Cell division</td>
<td>363</td>
<td>51</td>
<td>1.94E-29</td>
<td>8.32E-27</td>
</tr>
<tr>
<td>GO biological process</td>
<td>DNA repair</td>
<td>121</td>
<td>37</td>
<td>2.62E-21</td>
<td>1.13E-18</td>
</tr>
<tr>
<td>Chromosome arms</td>
<td>12q</td>
<td>693</td>
<td>88</td>
<td>4.72E-19</td>
<td>2.08E-17</td>
</tr>
<tr>
<td>Chromosome arms</td>
<td>5q</td>
<td>669</td>
<td>69</td>
<td>7.65E-11</td>
<td>3.38E-09</td>
</tr>
<tr>
<td>MSigDB, Functional sets</td>
<td>SERUM - FIBROBLAST - CELL CYCLE a</td>
<td>136</td>
<td>87</td>
<td>1.96E-83</td>
<td>3.31E-80</td>
</tr>
<tr>
<td>MSigDB, Regulatory-motif sets</td>
<td>E2F trans factor</td>
<td>192</td>
<td>41</td>
<td>3.51E-17</td>
<td>2.94E-14</td>
</tr>
</tbody>
</table>

Transcriptome-based Grading of ACC

- Transcriptome-based grade reflects mitotic grade
  - Cluster 1
    - Poor outcome
    - 14/16 high grade
    - 2/16 low grade
  - Cluster 2
    - Better outcome
    - 6/17 high grade
    - 11/17 low grade
TCGA: Pan-genomic grade also reflects proliferation

Emerging Molecular Classification

Histologic & Molecular Agreement on the Important Role of Proliferation

Optimal Way to Assess Proliferation?

Many studies on role of Ki67 in ACC diagnosis and grading

Duregon Study

**TCGA: Pan-genomic grade also reflects proliferation**

Cancer Cell 2016:29;723-36.

**Emerging Molecular Classification**


**Histologic & Molecular Agreement on the Important Role of Proliferation**

Low grade ACC  High grade ACC

**Optimal Way to Assess Proliferation?**

- Mitotic rate
  - Tedious and somewhat subjective
  - See many transfer cases where it is not done
- Molecular and genomic approaches
  - Too costly and impractical for routine work
- Ki67 immunohistochemistry
  - Widely available using established protocols

**Many studies on role of Ki67 in ACC diagnosis and grading**

- Susano et al. Mod Pathol 1985
- Babinska et al., 2008
- Weissferdt et al. Appl Imm Mol Morph 2014
- Duregon et al. Mod Pathol 2014
- Beuschlein et al. JCEM 2015
- Papathomas et al. AJSP 2016
- Simon et al. Surgery 2017

**Duregon Study**

Comparative diagnostic and prognostic performances of the hematoxylin-esin and phospho-histone H3 mitotic count and Ki-67 index in adrenocortical carcinoma

- 52 ACC cases from Turin
- 2 reviewers
  - Manual and computer-assisted methods
Mitotic Rate

PHH3 IHC

Ki67 IHC

Mod Pathol 2014:27;1246-54.

Three class grading of ACC by Ki67

LogRank (Mantel Cox): p<0.0001
- 20% vs 20-50%: p=0.072;
- 20-50% vs >50%: p=0.027;
- Median survival:
  - <20%: 1.5 months;
  - 20-50%: 47 months;
  - >50%: 15 months

Best prognostic indicator

Univariable Analysis of German Cohort

Table 1: Univariable Analysis (Cox Regression) of the German Cohort (n = 339)

<table>
<thead>
<tr>
<th>Factor</th>
<th>N of 339</th>
<th>RFS</th>
<th>HR</th>
<th>P Value</th>
<th>OS</th>
<th>HR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHH3 IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki67 IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kaplan-Meier Analysis of Ki67 on RFS and OS

RFS

OS

Multivariable Analysis

Table 4: Multivariable Analysis (Cox Regression) for ENSAT Stage and for Other Most Relevant Factors for RFS

<table>
<thead>
<tr>
<th>Factor</th>
<th>N of 339</th>
<th>RFS</th>
<th>HR 95% CI</th>
<th>P Value</th>
<th>OS 95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSAT stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHH3 IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki67 IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: “strong evidence that Ki67 index is the most powerful tool of all parameters analyzed in this study to predict recurrence…”
Strengths & Limitations of ENSAT Ki67 Study

- Large cohorts
- ENSAT database
- Compared continuous Ki67 values with binned mitotic rates
  - >5/50 hpf or >2/10 hpf
  - Mitotic rate did not have an equal chance to compete with Ki67
- Still an unanswered question

Ki67 Reproducibility Study

An International Ki67 Reproducibility Study in Adrenal Cortical Carcinoma

Thomas G. Papahadjopoulos, MD,*† Eugenio Priscu, MD,*† Thomas J. Goodwin, MD, PhD,*† Wan Lu, PhD,* EllenCausewicz, MD,*† Mario Volanti, MD, PhD,*† Matteo Pagani, MD,*† Ricardo V. Leibov, MD, PhD,*† Arthur S. Tischler, MD,** Francesco I. van Henderen, MD, PhD,†† Yvette van der Put, MD, PhD,‡‡ Jan F. van de Vijver, PhD,‡§ and Steven R. de Krijger, MD, PhD*††***

- 101 ACC from Italy, Netherlands, Wisconsin and Michigan
- IHC performed at single center (Erasmus)
- Evaluated by group of pathologists and by digital methods

Ki67 Reproducibility Study

Significant Variance between 14 observers using method of choice

Different cutoffs - OS

Challenges of Ki67 IHC

- Inter-laboratory variation
- Observer variation
- No agreement on appropriate cutoffs
- International Ki67 in Breast Cancer Workshop in 2011
  - No consensus on cutoffs
  - Need validation of local practice
  - Very difficult for ACC due to low incidence

Challenges in Assessing Proliferation in Adrenal Cortical Tumors

- Intra-tumoral heterogeneity
- Adenoma to carcinoma progression
- Admixture of low-grade and high grade components
- Molecular heterogeneity
Large tumors with ample opportunity for evolution

20 cm tumor

Intra-tumoral Heterogeneity

Despite all the limitations and challenges of assessing proliferation in adrenal cortical tumors,

I do it routinely.

Standard Workflow for Adrenal Tumors

- Generous tumor sampling
  - Including normal gland, tumor capsule and any displaced neoplasm to find pre-existing adenoma
- Routine histology
  - Adrenal IHC markers if needed to determine cortical differentiation
  - Diagnostic algorithm when needed
  - Identification of the highest grade component
  - Mitotic rate
  - IHC evaluation
    - Ki67 with image analysis (Ventana iScan Coreo slide scanner)
    - Beta-catenin and p53
    - Mismatch repair proteins for Lynch syndrome screening

Lynch ACC Case, MSH6 mutation

Incidence of 3%, same as in CRC, so should screen all ACC cases

Lynch ACC Case, MSH2 mutation

Recently discovered Lynch Syndrome case, MSH2 mutation verified by germline DNA sequencing
Adrenal cortical adenoma

No or few mitoses
Ki67 1-2%

IGF2 overexpression

Adrenal cortical carcinoma, low-grade

MI <20/50 hpf
Ki67 <15%

TP53 dominant
Copy number alterations

Adrenal cortical carcinoma, high-grade

MI >20/50 hpf
Ki67 >30%

CTNNB1 dominant
Copy number alterations

THANK YOU
&
Thanks to the Michigan Pathology Department
- Colleagues
- Jay Hess
- Jeff Myers
- Chuck Parkos