NEXT GENERATION LEARNING

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Dr. Ronald A. Ghossein declares he has no conflict(s) of interest to disclose.
Genomic landscape of poorly differentiated and anaplastic thyroid carcinomas: Clues for better classification, risk stratification and therapy

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Outline:

• Importance
• Histopathology:
  - Poorly differentiated carcinomas.
• Genomic landscape of poorly differentiated and anaplastic carcinomas.
  - Mutation burden
  - Somatic mutation
  - Gene fusion
  - Somatic copy number alterations
  - Gene expression
Importance of poorly differentiated and anaplastic carcinoma

- Rare (5-10%)

- Deadly (5 year survival: 60% poorly diff; 0-14% for anaplastic).

- No effective treatment.

- Comprehensive genotyping lacking and needed for novel therapies
“Old clinicians used to say that the classification of thyroid cancer was very simple. There was a group of well differentiated, slow growing tumors that never killed anybody, and a group of rapidly growing tumors that killed everybody”

L. Woolner

Dept. of Pathology

Mayo Clinic
Poorly Differentiated Thyroid Carcinomas

- Tumors of follicular cell origin showing histologic and prognostic features intermediate between Well Differentiated Thyroid Carcinomas and Anaplastic Carcinoma.
“Wuchernde struma”
P. Langhans
1907

Insular carcinoma
Carcangiu, Zampi, Rosai
1984
HISTOLOGIC FEATURES OF POORLY DIFFERENTIATED THYROID CARCINOMAS

- Solid/trabecular/insular growth
- Necrosis
- Capsular invasion
- Vascular invasion

*If all the above are present, everybody agrees on the Poorly differentiated diagnosis*
THE BIG QUESTION

- WHAT DEFINES POORLY DIFFERENTIATED THYROID CARCINOMAS?

- SOLID GROWTH PATTERN ALONE

OR

- MITOSIS/NECROSIS ALONE
TURIN PROPOSAL:
DIAGNOSTIC CRITERIA FOR PD CARCINOMA

Turin proposal: Diagnostic algorithm for poorly differentiated carcinoma

Necrosis and mitosis: Essential diagnostic criteria

“POORLY DIFFERENTIATED THYROID CARCINOMAS: DEFINED ON THE BASIS OF MITOSIS AND NECROSIS. A clinico-pathologic study of 58 cases.

Cancer (March) 2006.
POORLY DIFFERENTIATED THYROID CARCINOMAS

MSKCC DEFINITION

• Any tumor showing follicular cell differentiation at the histologic and immunohistochemical level (TGB +) with 5 or more MITOSIS per 10 high power fields and/or NECROSIS.

• Growth pattern and cell type (follicular or papillary) IRRELEVANT
Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis.
Overall survival
Poorly differentiated thyroid ca

Total number of patients=58
Number of deaths= 22
Median OS: 79 months
5 year survival: 60%
Predictors of poor overall survival in poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis

- Tumor > 4cm \( p=0.02 \)
- Absence of a capsule \( p=0.001 \)
- Extra-thyroid extension \( p=0.001 \)
- Margins \( p=0.001 \)
Factors with NO influence on overall survival in poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis

- Growth pattern (solid vs foll/pap) \( p=1 \)

- Cell type (oncocytic vs non-oncocytic) \( p=0.6 \)

- Cell size (large vs small) \( p=0.07 \)
POORLY DIFFERENTIATED THYROID CARCINOMA MAIN CAUSE OF RADIOACTIVE IODINE (RAI) REFRACTORY DISEASE

- 46% of radioactive iodine refractory PET positive thyroid carcinomas are poorly differentiated (based on mitosis and necrosis).

Many RAI refractory thyroid carcinomas with mitosis and necrosis are diagnosed as Papillary carcinoma or follicular carcinoma suggesting indolent behavior to clinicians.

- 15 (68%) of 22 poorly differentiated RAI refractory were initially classified as PTC, follicular carcinoma or Hurthle cell carcinoma.
INITIAL DX AND GROWTH PATTERN OF 15 RECLASSIFIED POORLY DIFFERENTIATED THYROID CARCINOMAS

<table>
<thead>
<tr>
<th>Initial Diagnosis</th>
<th>Growth Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Classical PTC (n=5)</td>
<td>1. 80% percent with papillary/follicular growth</td>
</tr>
<tr>
<td>2. PTC, follicular variant (n=2)</td>
<td>2. 100% with follicular growth</td>
</tr>
<tr>
<td>3. PTC, moderately differentiated (n=3)</td>
<td>3. 33% with follicular growth</td>
</tr>
<tr>
<td>4. Follicular/ Hurthle Cell Ca (n=4)</td>
<td>4. 50% with follicular growth</td>
</tr>
<tr>
<td>5. Diffuse sclerosing PTC (n=1)</td>
<td>5. Solid growth with clear nuclei</td>
</tr>
</tbody>
</table>
Genetic alterations found in advanced thyroid cancers so far

Poorly Differentiated Thyroid Cancers (PDTC)
Anaplastic Thyroid Cancers (ATC)

- **RAS**
  - Manenti et al 1994
- **TP53**
  - Donghi et al 1993
- **CTNNB1**
  - Garcia-Rostan et al 2001
- **BRAF**
  - Nikiforova et al 2003
  - Soares et al 2004
- **RET**
  - Santoro et al 2002
- **AKT1**
  - Ricarte-Filho et al 2009
- **PIK3CA**
  - Ricarte-Filho et al 2009
- **PTEN**
  - Pita et al 2014
- **TERT**
  - Liu-X et al 2013
  - Landa et al 2013
  - Vinagre et al 2013
- **EIF1AX**
  - Kunstman et al 2015
Latest comprehensive genomic analysis using next generation technology

• Kuntsman et al. Characterization of the mutational landscape of anaplastic thyroid cancer via whole exome sequencing. Hum Mol Genet 2015 (Yale)

## MSKCC vs Yale

<table>
<thead>
<tr>
<th></th>
<th>MSKCC</th>
<th>YALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor samples</td>
<td>84 poorly diff 33 anaplastic</td>
<td>No poorly diff 22 anaplastic</td>
</tr>
<tr>
<td>DNA sequencing</td>
<td>341 cancer gene</td>
<td>Whole exome</td>
</tr>
<tr>
<td>DNA sequencing coverage</td>
<td>500 x paraffin 765x frozen tissue</td>
<td>264 x</td>
</tr>
<tr>
<td>Additional testing</td>
<td>Array CGH</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Expression array</td>
<td></td>
</tr>
</tbody>
</table>
Mutation burden increase along the spectrum of thyroid progression

- Median No of mutation in 341 cancer genes:
  - Papillary carcinoma (TCGA): 1
  - Poorly differentiated: 2
  - Anaplastic: 6

p<001
Real mutation burden using whole sequencing

- **Anaplastic**: Mean: 89 /tumor
# Mutation burden and clinicopathological features in PDTC

**PDTC = 78**

<table>
<thead>
<tr>
<th></th>
<th>Below median (26)</th>
<th>Median (24)</th>
<th>Above median (28)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>47±15</td>
<td>58±15</td>
<td>64±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4</td>
<td>64%</td>
<td>57%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>36%</td>
<td>43%</td>
<td>71%</td>
<td>0.038</td>
</tr>
<tr>
<td>Pathology staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/T2</td>
<td>17%</td>
<td>15%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>T3/T4</td>
<td>83%</td>
<td>85%</td>
<td>96%</td>
<td>0.405</td>
</tr>
<tr>
<td>Nx/N0</td>
<td>54%</td>
<td>45%</td>
<td>52%</td>
<td></td>
</tr>
<tr>
<td>N1a/N1b</td>
<td>46%</td>
<td>55%</td>
<td>48%</td>
<td>0.822</td>
</tr>
<tr>
<td>M0</td>
<td>73%</td>
<td>54%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>8%</td>
<td>29%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Mx</td>
<td>19%</td>
<td>17%</td>
<td>11%</td>
<td>0.002</td>
</tr>
<tr>
<td>Overall survival (died)</td>
<td>19%</td>
<td>25%</td>
<td>46%</td>
<td>0.07</td>
</tr>
<tr>
<td>Overall survival time (days±SD)</td>
<td>2242±1332</td>
<td>2181±1406</td>
<td>1469±1158</td>
<td>0.05</td>
</tr>
<tr>
<td>Survival analysis: HR (95%CI)</td>
<td>HR:2.03</td>
<td>(1.19-3.47)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Log rank</td>
<td></td>
<td></td>
<td></td>
<td>0.014</td>
</tr>
</tbody>
</table>

**Kaplan-Meier survival estimates in PDTC**

Log-rank p = 0.014
### Somatic mutation frequency variable in the literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Anaplastic No mutated cases/total (%)</th>
<th>Poorly differentiated No mutated cases /total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nikiforova et al, 2003</td>
<td>3/29 (10.3%)</td>
<td>2/16 (12.5%)</td>
</tr>
<tr>
<td>Soares et al, 2004</td>
<td>6/17 (35.3 %)</td>
<td>0/19 0</td>
</tr>
<tr>
<td>Garcia Rostan et al, 2005</td>
<td>19/69 (27.5%)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Santarpia et al, 2008</td>
<td>2/18 (11.1 %)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Liu et al, 2008</td>
<td>14/50 (28%)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Costa et al, 2008</td>
<td>9/36 (25%)</td>
<td>4/24 (16.7%)</td>
</tr>
<tr>
<td>Ricarte-Filho et al, 2009</td>
<td>8/18 (44.4%)</td>
<td>4/34 (11.8%)</td>
</tr>
<tr>
<td>Pita et al, 2014</td>
<td>2/26 (7.7%)</td>
<td>1/22 (4.5%)</td>
</tr>
<tr>
<td>Kuntsman et al, 2015</td>
<td>6/22 (27.3%)</td>
<td>N/A N/A</td>
</tr>
<tr>
<td>Landa et al, 2016</td>
<td>15/33 (45.5%)</td>
<td>28/84 (33%)</td>
</tr>
</tbody>
</table>
Why variable frequency?

- Technical difference (MSKCC platform more sensitive than whole exome)
- Macrophage infiltration in advanced tumor (anaplastic)
Anaplastic carcinomas are heavily infiltrated by macrophages.
81% of BRAF

PDTC definition

MSKCC high mitotic rate and necrosis irrespective of growth pattern

92% of RAS

Turin proposal solid growth plus high grade features (mitosis and necrosis)

**EIF1AX**, a component of the translation initiation machinery, is mutated in 10% of PDTCs and ATCs

**PIK3CA** and **PTEN** are frequent events in ATC, specific patterns:

- **PIK3CA-BRAF**
- **PTEN-NF1**
**EIF1AX: RAS association and prognostic value**

*EIF1AX mutations*

- **Anaplastic thyroid cancers** (Kunstman et al, 2015)
  - EIF1AX-RAS association: 14/15 PDTCs+ATCs
  - 3/3 Cell lines
  - 3/3 ATCs from Kunstman et al
  - OR= 58.3
  - Log-rank $p = 0.048$

- **Advanced thyroid tumors** (MSKCC)
  - EIF1AX: 10%

- **Papillary thyroid cancers** (TCGA-PTC)
  - EIF1AX: 1%

**Other tumors**

- **COSMIC**
  - missense mutation
  - splice site mutation
  - frameshift deletion
  - nonsense mutation

- **Uveal melanoma**

**Papillary thyroid tumors (TCGA)**

- EIF1AX: 1%

**Poorly-differentiated thyroid tumors (MSKCC)**

- EIF1AX: (MSKCC, n=33) + (Kunstman et al, 2015, n=22)
  - EIF1AX: 11%

**Anaplastic thyroid tumors (n=55)**

- EIF1AX: 11%
  - BRF: 38%
  - NRAS: 16%
  - HRAS: 10%
  - KRAS: 4%

*EIF1AX-RAS association*

- 14/15 PDTCs+ATCs
- 3/3 Cell lines
- 3/3 ATCs from Kunstman et al

OR= 58.3

Log-rank $p < 0.0001$
**TERT** promoter mutations increase in advanced thyroid cancers and co-occur with RAS and BRAF mutations

**Papillary thyroid tumors (TCGA)**
- **TERT**: 9%
- **BRAF**: 56%
- **NRAS**: 0%
- **HRAS**: 4%
- **KRAS**: 1%

**Poorly-differentiated thyroid tumors**
- **TERT**: 40%
- **BRAF**: 33%
- **NRAS**: 21%
- **HRAS**: 5%
- **KRAS**: 2%

**Anaplastic thyroid tumors**
- **TERT**: 73%
- **BRAF**: 45%
- **NRAS**: 18%
- **HRAS**: 6%
- **KRAS**: 0%

**TERT-BRAF/RAS association**
- **OR**: PTC 3.3, PDTC+ATC 3.4
- **p-value**: 0.03, 0.004

**Diagram:**
- **RTK** via MAPK pathway
- **RAS** → **BRAF** → **MEK** → **ERK**
- **TERT** promoter mutations
- **TERT-BRAF/RAS association**
- **PTC** vs. **PDTC+ATC**
- **OR**: 3.3, 3.4
- **p-value**: 0.03, 0.004
TERT promoter mutations in thyroid cancers

TERT promoter mutations are subclonal events in PTCs, but clonal in PDTCs and ATCs → key transitional event in tumor microevolution
P53 and other tumor suppressor genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Poorly differentiated (%)</th>
<th>Anaplastic (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>8%</td>
<td>73%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ATM</td>
<td>7%</td>
<td>9%</td>
<td>ns</td>
</tr>
</tbody>
</table>
PATHWAYS:
- Enriched in mutations in ATC, PDC vs PTC (1-4%)
- Mutations more frequent in ATC vs PDC (p<0.05)
WNT signaling pathway (B catenin, APC, axin1)

• >60% frequency in anaplastic ca in the literature.

• MSKCC 2016: 3% of anaplastic

• Yale 2015: 4.5% of anaplastic ca
Somatic arm-level copy number alterations (CNAs)

- **Tumor type**
  - Genetic driver
  - Tumor purity

- **Copy number (log-ratios)**
  - Gain: +1.5, +0.9, +0.3
  - Loss: -0.3, -0.9, -1.5

- **Tumor type**
  - Poorly-differentiated thyroid cancers
  - ATCs

- **Genetic driver**
  - BRAF
  - RAS
  - Fusion

**Key Observations**

- **20q gains in ATCs**
- **22q losses in RAS-mutant PDTCs**
Somatic CNAs are associated with clinical outcome.

**chr1q gains in PDTC**
- Diploid gain
- Log-rank p = 0.03

**chr13q losses in ATC**
- Diploid loss
- Log-rank p = 0.07

**chr20q gains in ATC**
- Diploid gain
- Log-rank p = 0.01
Transcriptomic differences in PDTC vs. ATC

Principal component analysis separates PDTCs and ATCs based on their global gene expression.

A signature of genes overexpressed in M2-macrophages (Coates, 2008) is sufficient to discriminate ATCs from PDTCs.
Poorly differentiated carcinomas signal according to their BRAF/RAS mutation whereas anaplastic tend to be BRAF-like (higher MAPK output) regardless of their driver.
Anaplastic are profoundly undifferentiated compared to poorly differentiated carcinoma.
What did we learn?

- Step wise progression from well diff to poorly diff to anaplastic further confirmed.

- TERT promoter mutations may identify subset of PTC at risk of progression.

- Sharp clinico-pathologic demarcations between \textit{BRAF} and \textit{RAS}-mutant disease persists in Poorly diff but are largely lost in Anaplastic ca. (genomic complexity of anaplastic)
What did we learn?

- Key genetic events differentiating poorly diff from anaplastic identified (p53, TERT, PIK3CA pathway, novel (SWI/SNF, histone methyltransferase)
- Copy number alterations distinctive of anaplastic and poorly diff ca
- Potential prognostic markers within poorly diff (EIF1AX, Mutation burden, 1q gain)
What did we learn?

• Potential prognostic markers within anaplastic (EIF1AX, 13q loss, 20q gain).

• Opportunity to explore the biology of novel genetic associations for therapy.
THE END