Progressive Thyroid Cancer: Defining Targets and Designing Therapies

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Case

- 45 year old woman diagnosed with a 3.5 cm angioinvasive insular variant of follicular thyroid carcinoma in 2003.
- She received 160 mCi for remnant ablation following thyroxine withdrawal.
- Post-therapy scan with uptake in the thyroid bed
- Tg <0.9 ng/ml at time of therapy with anti-Tg antibodies 45 IU/mL

Case

- 2004: rhTSH I-131 4 mCi scan
  - Stimulated Tg was <0.9 with negative anti-Tg antibodies
  - WBS had no uptake
- April, 2005: rhTSH Tg is 4.3 ng/mL
  - I-131 scan is negative
  - PET/CT with uptake in the right submandibular region node
  - Chest CT without contrast with several small pulmonary nodules
- Surgery in the right neck revealed a benign node

Case

- June, 2006
  - L-T4 withdrawal was performed:
    - Tg was 10.8 ng/ml
    - 150 mCi I-131 with negative post-therapy scan
- Chest CT with increased size of lesions
- Wedge resection: Insular thyroid cancer, Tg positive and similar appearance to primary thyroid cancer.
Case

- April, 2007: Referred for evaluation
  - Normal Exam
  - TSH:<0.04 mU/L Free T4: 1.87 ng/dl; Tg: 10.9 ng/ml with negative anti-Tg antibodies.
  - Chest CT stable vs 2006

Case 3

<table>
<thead>
<tr>
<th>Date</th>
<th>TSH (mU/L)</th>
<th>Tg (ng/ml)</th>
<th>Tg Ab (IU/ml)</th>
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<td>&lt;.04</td>
<td>10.9</td>
<td>&lt;20</td>
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<td>&lt;.04</td>
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<td>&lt;.04</td>
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<td>200</td>
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PET/CT repeated in 9/07
- New lesion in hypopharynx: SUV 4.4
- Pulmonary nodules: stable since last CT. One with mild uptake with SUV 1.2 in left lower lobe. Largest nodule 1.0 cm in left lower lobe.
- Surgical removal of hypopharynx lesion was benign inflammatory tissue.
Case

- Chest CT repeated in 1/08
  - Progression of left lower lobe nodule: now 2.6 cm.
  - Right nodule now 1.4 cm that was 0.8 cm and stable.

- What would you do now?

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Case: Progressive Iodine non-responsive Thyroid Cancer

- “Referral for participation in clinical trials should be considered for patients with progressive or symptomatic metastatic disease. For those patients who do not participate in clinical trials, treatment with tyrosine kinase inhibitors should be considered.” Grade Level B

Cooper DS, et al. Thyroid 2009

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Thyroid Cancer Oncogenes

- RET
- cMET
- VEGFR
- PDGFR
- EGFR
- IGFR
- Many others

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Potential Kinase Targets in Thyroid Cancer

- Receptors
  - RET
  - cMET
  - VEGFR
  - PDGFR
  - EGFR
  - IGFR

- Signaling Kinases
  - BRAF
  - AKT
  - MEK
  - PI3K
  - Many others
Non-Kinase Targets for Progressive Thyroid Cancer

- Histone Acetylation (HDAC inhibition)
- DNA hypermethylation
- Protein Stability
  - Chaperone molecule inhibitors (Hsp90)
  - Proteasome Regulators
- Vascular-targeted therapies
- Immunological-related Therapies
- Combinations with Cytotoxic Chemotherapies

Thyroid Cancer U.S. Clinical Phase 2/3 Trials Currently Recruiting: 6/16/10
(www.clinicaltrials.gov)

<table>
<thead>
<tr>
<th>DTC</th>
<th>DTC/ATC</th>
<th>ATC</th>
<th>MTC</th>
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<tbody>
<tr>
<td>Sunitinib</td>
<td>Sorafenib</td>
<td>Combrertatin/paclitaxel</td>
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<td>E7080</td>
<td>Pazopanib</td>
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<td>Paopanib</td>
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<td>Axitinib</td>
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<td>Aflibercept</td>
<td>Vandetanib</td>
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<td>Fostamatinib</td>
<td>Vandetanib + Bortezomib</td>
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<td>Sunitinib + I-131</td>
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<tr>
<td>AZD6244 + I-131</td>
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<td>Lithium</td>
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Tyrosine Kinase Inhibitors in Differentiated Progressive Thyroid Cancer: Clinical Trials

Table 1: Kinase inhibitors recently in clinical trials for advanced or metastatic thyroid carcinomas

<table>
<thead>
<tr>
<th>Drug category/Drug</th>
<th>VEGFR1 IC50(μM)</th>
<th>VEGFR2 IC50(μM)</th>
<th>VEGFR3 IC50(μM)</th>
<th>RET IC50(μM)</th>
<th>MET/PTC3 IC50(μM)</th>
<th>BRAF IC50(μM)</th>
<th>Other IC50(μM)</th>
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<td>Axitinib</td>
<td>1.2 0.25 0.29</td>
<td>C-KIT 1.7 (23)</td>
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<tr>
<td>Gefitinib</td>
<td>EGFR 33 (57)</td>
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<tr>
<td>Sorafenib</td>
<td>I-131 (54)</td>
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<tr>
<td>Sunitinib + I-131</td>
<td>E7080</td>
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</tr>
<tr>
<td>Motesanib</td>
<td>2 3 6 59</td>
<td>C-KIT 8 (16)</td>
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<tr>
<td>Sorafenib</td>
<td>90 20 47 50 22</td>
<td>C-KIT 68 (36)</td>
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<tr>
<td>Sunitinib</td>
<td>2 9 17 41 224 (46)</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Vandetanib</td>
<td>1000 40 110 130 100</td>
<td>EGFR 500 (28 29)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>XL184</td>
<td>0.035 130 100</td>
<td>C-MET 1.8 (64)</td>
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</table>

VEGFR, EGF receptor.
Case

- Clinical trial enrollment delayed
  - Did not qualify for one due to H/O psvt still treated with digoxin
  - Referred for a second trial that closed prior to her enrollment.
- 4/08: Rising Tg.
- Repeat CT with new 2 and 4 cm paratracheal nodes
- Repeat PET/CT with increase in hypermetabolic lung lesions with the 2 new masses being positive.
- Offered local XRT and then sorafenib off-trial

Case

- Pt underwent XRT
- Sorafenib started and pt had a radiographic and biochemical partial response to sorafenib for 9 months
- Gradually escaped from effect and developed increased side effects
- Pathological Hip fracture
- Now on Carboplatinum/Taxol with partial response

Targeting Thyroid Cancer Therapy: Patient Selection for Clinical Trials and New Directions

- Patients that are appropriate for non-standard therapies for thyroid cancer have surgical and iodine non-responsive and progressive thyroid cancer, or very poorly differentiated tumors.
- Determine the cells/tissues being targeted and identifying the functional targets of the compounds.
- Identify mechanisms of resistance
- Devise Combination therapies and/or new therapeutic targets.
Targeting Therapies for Thyroid Cancer

- Differentiated
- Progressive
- Anaplastic

| TSH-Receptor Na, I Symporter Oncogene Progression Targets |

Stages of Tumor Progression

- Primary Tumor Growth & Invasion
- Vascular Invasion
- Macrophage Recruitment & Endothelial and Stromal Activation
- Metastatic Progression
- Reinvasion & Growth

Mechanisms of Papillary Thyroid Cancer Invasion

- Signaling Pathways in Thyroid Cancer Progression
- Global Analysis of Papillary Thyroid Cancer Invasion
- P21 Activated Kinase in Thyroid Cancer

Global Assessment of Invasion in Thyroid Cancer

- 7 widely invasive PTCs were microscopically dissected into central and invasive regions comprised of >90% thyroid cancer cells. RNA was also isolated from normal tissue.
- Affymetrix Chips (U133b) were used to evaluate the expression profiles of paired normal, central tumor, and invasive tumor regions from individual patient.
- Gene expression was compared between normal and central tumor and central tumor and invasive regions for each patient.
- Genes that were differentially expressed between central and normal; and invasive and central were identified.

Analysis and confirmation of oligonucleotide expression microarrays

EASE Functional Analysis: Center of Tumor Vs Normal

- Overexpressed genes corresponded to:
  - Cell-cell adhesion
  - Cell-cell communication
  - Extracellular Proteins and peptidase activity
  - Proliferation
- Reduced in Central Tumors
  - Heavy Metal and cation homeostasis
  - Immune response and antigen presentation
  - Chemotaxis

EASE functional analysis invasion vs central

- Overexpressed genes in the invasive regions of the PTCs:
  - transcription, due largely to an increase in expression of zinc finger DNA binding proteins;
  - Signaling, including genes in the Integrin and TGFβ pathways such as TGFBR2, SMADs, and RAF1;
  - genes regulating nuclear localization of proteins, such as XPO, nucleoporins, dynamin, and kinesin;
  - small G-proteins, guanine nucleotide exchange factors and downstream signaling molecules including KRAS, RAC2, RHGEF7, and RND2.
- Down regulated gene in the invasive regions of the PTCs:
  - cell-cell adhesion and communication
    - These functions were increased in the central versus normal suggesting a specific loss of cell adhesion in the invasive areas.

Epithelial-to-Mesenchymal Transition (EMT)

- The microarray functional analysis suggested that the invasive regions of PTC might have EMT
- EMT is defines as when epithelial cells morphologically and behaviorally change and develop characteristics of mesenchymal cells including:
  - Fibroblastoid shape
  - Increased Motility
  - Expression and organization of intermediate filaments such as desmin and vimentin
  - Reduced contact inhibition
- EMT or partial EMT has been implicated to be functionally important in the process of cancer invasion and metastasis.
- Master regulators of EMT include: Snail, Twist, Vimentin, TGF beta and PAK.
Validation Series: Is EMT a predictor of Invasion or Metastasis in PTC?

- 34 PTC Samples
  - 20 with invasion
  - 24 with nodal metastasis
- Vimentin was chosen for this study as a downstream marker of EMT that was not overexpressed in the microarrays
- RUNX2 was also examined to confirm the microarray data due to its proposed role in EMT
- IHC was scored independently by three investigators on a 0-3 scale
- High Levels of Vimentin were associated with:
  - Tumor invasion (p<0.001)
  - Tumor multifocality (p<0.01)
  - Nodal Metastasis (p<0.005)
  - High levels of RUNX2 (p<0.02)
- Vimentin Overexpression occurred independent of Oncogene
- These data suggest that EMT is a common event in PTC invasion.


Expression of mesenchymal proteins in PTC invasion is independent of oncogene

Vasko V. et.al. PNAS 2007;104:2803-2808

Vimentin and Osteopontin are Increased in Thyroid Cancer Invasion

Gene Expression Profiles of Primary PTC Invasive Fronts

- EMT-like profile was overrepresented in the invasive fronts.
- Bioinformatics suggested that cdc42-PAK signaling may be an important signaling node.
- EMT processes were common in invasive PTCs independent of the initiating oncogene.
- Is PAK a functional target for invasive PTCs?

Thyroid Cancer Signaling and PAK Activation

PI3K

PIP2 → PIP3

PDK1 → AKT

mTOR

Apoptosis

PAK

Rac CDC42

PAK Structure

Group I: PAK 1,2,3 (Classical): Inducible Activity

N-terminal → PBD → AID → PIX binding → Kinase → C-terminal

Grb2

Group II: PAK 4,5,6: Constitutive Activity

N-terminal → PBD → Kinase → C-terminal

PAK: p21 binding domain
AID: auto inhibitory domain
Nck and Grb2: SH3
PIX binding: non-classical SH3

Thyroid Cancer Cell Motility is Group I PAK-Dependent
Thyroid Cancer Cell Migration is PAK1-Dependent

![Graph showing Thyroid Cancer Cell Migration](image)

Downstream Targets of PDK1

![Diagram showing Downstream Targets of PDK1](image)

OSU03012, a novel PDK1 inhibitor

- Based on Structure of Celecoxib
  - No COX inhibitory Activity
  - Competitively blocks PDK-1 kinase
  - IC50 ~5 uM
- Effective in PTEN null cell lines in vitro and in vivo.


OSU-03012 Directly Inhibits PAK

![Diagram showing OSU-03012 Directly Inhibits PAK](image)

Porchia, et al Mol Pharm. 2007
OSU-03012 Inhibits Cancer Cell Growth and PAK Signaling in vivo.

EMT and Thyroid Cancer Progression

- Epithelial-to-mesenchymal transition is common in invasive PTCs.
- EMT appears to be a common late-stage event that is independent of oncogene expression in this small series.
- Vimentin overexpression is associated with invasion and nodal metastases, but not tumor size, in primary PTCs.
- PAK represents an invasion-related signaling node that may play an important role in thyroid cancer invasion; thereby representing a potential therapeutic target.
- OSU-03012 includes PAK in its target profile and is in phase 1 clinical trials.

Summary

- Determining Regulatory Pathways in Thyroid Cancer progression at primary and metastatic sites represents a challenge with important therapeutic consequences.
- Current Agents inhibit progression and induce remission that is non-durable in ~50% of patients
  - Actual Targets of Agents are Uncertain
- Identifying key mechanisms of cancer progression remains a key goal in advancing therapeutic options for patients with progressive thyroid cancer.
- Tissues from progressive Distant Metastases are Needed
- Likely that combination therapies or “dirty” drugs ultimately will be needed for more effective therapy.
Collaborators

- Morris Bimbaum
- Leonard Kohn
- Judith Meinkoth
- Mike Tuttle
- Geraldo Medeiros-Neto
- Constantine Stratakis
- Alfredo Fusco
- Tom Giordano

- Charis Eng
- Sheue-Yann Cheng
- Danny Welch
- John Lannutti
- Mike Paulaitis
- Ileana Rubio
- Vasily Vasko
- Sylvia Asa
- Caroline Kim

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- Phay, John
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