Stromal reaction in medullary thyroid carcinomas

Oskar Koperek
Clinical Institute of Pathology
Medical University Vienna
Medullary Thyroid Carcinoma (MTC)

- derive from the Calcitonin producing C-cells of the thyroid gland
- 7 - 10% of all thyroid carcinomas
- 25% familial
- m:f = 1:1.3
- mostly located in the upper 2/3 of the thyroid gland
- may develop from c-cell hyperplasia
C-cell hyperplasia

- more than 50 cells per low power field (100x) in both thyroid lobes

- different types:
  - focal
  - diffuse
  - nodular
  - neoplastic

- precursor lesion in familial disease (sporadic cases?)
MTC - Metastasis

• up to 50% of MTC presents with lymph node metastasis (and in 10-30% medullary thyroid microcarcinoma!!)

• up to 20% distant metastasis
MTC – Therapy

- Curative Therapy:
  - Surgery
    - Total thyroidectomy + functional central and lateral neck dissection
MTC - Macroscopy

- yellow-white
- soft-firm
- well demarcated
MTC - Histology

- cells in nests and trabeculae
  - follicular variant
  - papillary variant
  - paragangliom-like variant
- polygonal, round, spindle shaped cells
  - small cell type
  - oxyphilic cell type
- desmoplastic stroma reaction
- amyloid
MTC

- amphophilic granulated cytoplasm (neuroendocrine vesicles)
- excentric round nuclei with coarse chromatin (salt and pepper)
- immunohistochemistry
  - Calcitonin
  - ev. CgA, CEA...
- DD: poorly differentiated thyroid carcinoma, paraganglioma, metastasis of a differentiated neuroendocrine neoplasm...
Sporadic Versus Familial Medullary Thyroid Microcarcinoma
A Histopathologic Study of 50 Consecutive Patients

Klaus Kaserer, M.D., Christian Scheuba, M.D., Nikolaus Neuhold, M.D., Andreas Weinhäusel, Ph.D., Oskar A. Haas, M.D., Heinrich Vierhapper, M.D., and Bruno Niederle, M.D.

Tumours with desmoplasia
• LN Metastasis in 8/33 patients

Tumours without desmoplasia
• no LN Metastasis (0/17)
Desmoplasia in medullary thyroid carcinoma: a reliable indicator of metastatic potential

O Koperek, C Scheuba,1 M Cherenko,2 N Neuhold,3 C De Micco,2 K W Schmid,4 B Niederle1 & K Kaserer

**DESMOPLASIA**

= newly formed fibrotic/collagenous stroma, that surround the invasive tumour cells not seen in the preexisting thyroid tissue.

<table>
<thead>
<tr>
<th>Tumours with desmoplasia</th>
<th>Tumours without desmoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN Metastasis (61/152)</td>
<td>no LN Metastasis (0/44)</td>
</tr>
<tr>
<td>pT1: 70, pT2: 64, pT3: 2, pT4: 16</td>
<td>pT1: 34, pT2: 10</td>
</tr>
</tbody>
</table>

TNM nach UICC 1997
Observer-Variability und Desmoplasia

- Intraobservervariability:
  - $\kappa = 0.883$ (5/120 discrepant)

- Interobservervariability:
  - $\kappa_{NN} = 0.837$, $\kappa_{CDM} = 0.79$, $\kappa_{KWS} = 0.758$
  - $\kappa_{Marseille} = 0.759$ (5/76 discrepant)

102/120 concordant cases (22 without desmoplasia and 80 with desmoplasia), 13 cases with one discrepant pathologist (seven without and six with demoplasia)

No case evaluated as „desmoplasia negative“ by at least one observer showed LN-metastasis!

$\kappa = $ Cohen`s kappa coefficient
Molecular characterisation of the stroma in MTC

Tenascin-C, extracellular matrix protein
- promotes cell-migration
- inhibits cell adhesion to fibronectin
- induces tumor growth and metastasis

Fibroblast activation protein α (FAPα), Typ 2 serin protease
- degrading of collagen I

Stroma remodelling in concomitant c-cell hyperplasia

Calcitonin  Tenascin C  FAP

Tenascin C in c-cell hyperplasia

Koperek O et al. Tenascin C in medullary thyroid microcarcinoma and C-cell hyperplasia. Virchows Archiv 2008
Stromal reaction in other thyroid carcinomas

- PTC
- FTC

FAP

TEN

PTC with LN-status

In silico expression-analysis
(BioExpress™ Databank, Gene Logic Inc., Gaithersburg, MD, USA)

Koperek et al.: Molecular characterization of the desmoplastic tumor stroma in medullary thyroid carcinoma. Int J Oncology 2007
Further question?

• What causes the desmoplastic stromal response in thyroid carcinomas?
Hypoxia

- Assoziation with metastasis
- Assoziation with fibrosis (and necrosis)

Couvelard A, O'Toole D, Leek R et al. Expression of hypoxia-inducible factors is correlated with the presence of a fibrotic focus and angiogenesis in pancreatic ductal adenocarcinomas. Histopathology 2005;46:668-676
Colpaert CG, Vermeulen PB, Fox SB et al. The presence of a fibrotic focus in invasive breast carcinoma correlates with the expression of carbonic anhydrase IX and is a marker of hypoxia and poor prognosis. Breast Cancer Res Treat 2003;81:137-147
Hypoxia inducible factor 1 alpha (HIF 1α)

- HIF1α is a subunit of the transcription factor HIF1.
- Under normoxic condition this protein is hydroxylated (HIF prolyl-hydroxylases), ubiquinised (VHL E3 ubiquitin ligase) and degraded within the cytoplasm.

Under hypoxic condition the hydroxylation is inhibited and the stabilized HIF1α is transported into the nucleus and with the HIF1b subunit and other cofactors it forms the transcription factor HIF1.

- HIF1 activates the glycolysis, the angiogenesis (VEGF) and regulates the tissue pH (via carbonic anhydrase IX),...
HIF-1α regulation by proline hydroxylation
Veronica A. Carroll and Margaret Ashcroft
Expert Reviews in Molecular Medicine: http://www.expertreviews.org/
Accession information: Vol. 7; Issue 6; 15 April 2005
Hypoxic reaction in MTC

- 100 cases of sporadic MTC and if available LN-metastasis

- Immunohistochemistry:
  - Hypoxic markers: HIF1α and Carbonic anhydrase IX
  - Stromal marker: tenascin C
  - Proliferation marker: ki-67 (MIB1)
    - (proliferation index by counting 1000 cells)
  - Cell adhesion marker: ecadherin
Carbonic anhydrase IX

- transmembrane protein
- pH-regulation
- available antibody for immunohistochemistry on formalin fixed paraffin embedded tissue
- its expression correlates with hypoxia

HIF 1α und Carbonic anhydrase IX in MTC

Immunoreactivity is seen in a part of the MTC

- focally accentuated (accept two cases with strong diffuse immunoreaction)

- Expression of hypoxic markers in LN-metastases is similar to the expression in the primary tumours
Mutation in the VHL-Gene

VHL gene, exon 1: Analyses of the sequencing chromatograms (No. 910_10) shows a point mutation in Codon 78 (AAT > ATT) 
SeqScape 2.5 (Applied Biosystems, Foster City, CA, USA).

VHL gene, exon 1: Mikrodeletion of three base pairs at Codon 76 (delTTC) 
Sequencing Analyses Software (Applied Biosystems, Foster City, CA, USA)

Rot: Thymin (T); blau: Cytosin (C); schwarz: Guanin (G); grün: Adenin
Correlations of hypoxia associated proteins with clinicopathologic parameters

<table>
<thead>
<tr>
<th></th>
<th>CA IX p-value</th>
<th>HIF 1α p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of positive cases (percentage)</td>
<td>number of positive cases (percentage)</td>
</tr>
<tr>
<td><strong>T - Staging</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT 1 (n = 57)</td>
<td>19 (33.3%)</td>
<td>17 (29.8%)</td>
</tr>
<tr>
<td>pT 2 (n = 29)</td>
<td>20 (69%)</td>
<td>21 (72.4%)</td>
</tr>
<tr>
<td>pT 3 (n = 2)</td>
<td>2 (100%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>pT 4 (n = 12)</td>
<td>12 (100%)</td>
<td>11 (91.7%)</td>
</tr>
<tr>
<td><strong>Multifocality</strong></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>yes (n = 17)</td>
<td>7 (41.2%)</td>
<td>9 (52.9%)</td>
</tr>
<tr>
<td>no (n = 83)</td>
<td>46 (55.4%)</td>
<td>42 (50.6%)</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>yes (n = 27)</td>
<td>21 (77.8%)</td>
<td>20 (74.1%)</td>
</tr>
<tr>
<td>no (n = 73)</td>
<td>32 (43.8%)</td>
<td>31 (42.7%)</td>
</tr>
<tr>
<td><strong>Desmoplasia</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 75)</td>
<td>47 (62.7%)</td>
<td>46 (61.3%)</td>
</tr>
<tr>
<td>no (n = 25)</td>
<td>6 (24%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td><strong>Extrathyroidal invasion</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 12)</td>
<td>12 (100%)</td>
<td>11 (91.7%)</td>
</tr>
<tr>
<td>no (n = 88)</td>
<td>41 (46.6%)</td>
<td>40 (45.5%)</td>
</tr>
<tr>
<td><strong>Peritumoural invasion</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 53)</td>
<td>29 (54.7%)</td>
<td>26 (49.1%)</td>
</tr>
<tr>
<td>no (n = 47)</td>
<td>24 (51.1%)</td>
<td>25 (53.2%)</td>
</tr>
<tr>
<td><strong>Vascular invasion</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 8)</td>
<td>7 (87.5%)</td>
<td>5 (62.5%)</td>
</tr>
<tr>
<td>no (n = 92)</td>
<td>46 (50%)</td>
<td>46 (50%)</td>
</tr>
<tr>
<td><strong>mitosis</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 10)</td>
<td>5 (50%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>no (n = 90)</td>
<td>48 (53.3%)</td>
<td>47 (52.2%)</td>
</tr>
<tr>
<td><strong>Necrosis</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 6)</td>
<td>6 (100%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>no (n = 94)</td>
<td>47 (50%)</td>
<td>46 (48.9%)</td>
</tr>
<tr>
<td><strong>Calcification</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 15)</td>
<td>12 (80%)</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>no (n = 85)</td>
<td>41 (48.2%)</td>
<td>41 (48.2%)</td>
</tr>
<tr>
<td><strong>Amyloid</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 47)</td>
<td>32 (68.1%)</td>
<td>32 (68.1%)</td>
</tr>
<tr>
<td>no (n = 53)</td>
<td>21 (39.6%)</td>
<td>19 (35.8%)</td>
</tr>
<tr>
<td><strong>Lymphocytes within the tumour</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>yes (n = 28)</td>
<td>17 (60.7%)</td>
<td>17 (60.7%)</td>
</tr>
<tr>
<td>no (n = 72)</td>
<td>36 (50%)</td>
<td>34 (47.2%)</td>
</tr>
<tr>
<td><strong>Inflammation in the preexisting thyroid tissue</strong></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>No (n = 67)</td>
<td>36 (53.7%)</td>
<td>35 (52.2%)</td>
</tr>
<tr>
<td>Little (n = 12)</td>
<td>6 (50%)</td>
<td>8 (66.7%)</td>
</tr>
<tr>
<td>moderate/prominent (n = 21)</td>
<td>11 (52.4%)</td>
<td>8 (38.1%)</td>
</tr>
</tbody>
</table>
Correlations

Hypoxia associated proteins correlated significantly

• with the expression of tenascin C

• with the proliferation index (ki-67)
  mean index: 1% (range: 0.1% - 6.1%)

no significant correlation with the expression of ecadherin
HIF1a und CAIX in MTC

Summary

- Our findings suggest that despite of the fact that MTCs have only slowly growth, the upregulation of HIF1 pathway (hypoxia induced or oncogenetically) plays an important role in the development of loco-regional metastases and desmoplastic stromal reaction.

- Since traditional cytotoxic chemotherapy has only little effect on MTCs, targeting hypoxia associated and regulated proteins might be of benefit for patients with inoperable tumours.
Thank You!

Clinical Institute of Pathology, Medical University of Vienna (MUW):
Peter Birner
Oliver Bergner
Bettina Pichlhöfer
Felicitas Oberndorfer
Reinhard Horvat

Clinical Institute of Neurology, MUW:
Johannes A Hainfellner

Department of Surgery, MUW:
Bruno Niederle
Reza Asari
Christian Scheuba

Boehringer Ingelheim, Vienna:
Pilar Garin-Chesa
Oliver Bergner
Wolfgang Rettig
Christian Haslinger
Christine Puri

Private Laboratory Kaserer & Beer:
Klaus Kaserer

Department of Pathology and Bacteriology, Kaiserin Elisabeth Spital, Vienna:
Nikolaus Neuhold

Institute of Pathology and Neuropathology, University Essen:
Kurt Werner Schmid

Institute of Pathology, University Marseille:
Catharine De Micco
Maria Cherenko

Institute of Medical Oncology, Oxford:
Adrian L Harris