Future outlooks in NET:

The pathologists point of view

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ESMO Preceptorship on GI neuroendocrine tumors (NETs)
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Near Future

> Differentiation between NET G3 and NEC G3

> Predictive markers
Molecular sub-Classification of NET?

> Breast cancer, protein expression, expression profile

> Colon cancer, mutations and pathways?

> Lung cancer, mutations

> Melanomas, mutations and inflammation?

> NET: PT location and proliferation?
Looking at NET from Pathways: Possibilities
PCC, MTC, pNET (familial syndromes)

- Hypoxia (VHL, SDHx, Hif 2a, PHD3)
  — PCC/PGL, pNET, siNET

- mTOR activation (NF1, TSC2, PTEN)
  — pNET, siNET

- Epigenetic changes, Chromatin remodeling (Men1)
  — pNET, lungNET
Hypoxia (VHL, SDHx, Hif 2a, PHD3)

- PCC/PGL, pNET, siNET
- Hypoxia: lack of oxygen
- Pseudohypoxia: genetic/epigenetic changes induce signaling similar to hypoxia
Pseudohypoxia: PCC/PGL

Dahia, PloS Gen, 2005
Pseudohypoxia: PCC/PGL

Hypoxia, ECM, others

Cell-cycle
Pseudohypoxia: PCC/PGL

Eisenhofer, ERC, 2004
Pseudohypoxia: other NET?

> pNET VHL vs. sporadic:
  — Upregulation of HIF-targets (CA9, HIF2a, Glut1)
  — Angiogenesis (VEGFR1,2, VEGFA, CD34, EDNRA, ANGPT1,2)
  — EMT (Vim)
  — Metastasis related genes (LAMA4, CXCR4)
  — Growth factors (EGFR, PDGFR, IRS1)
  — Cell cycle (Cyclin D1)
  — Downregulation of EMT (OCLN)
  — Signaling pathway (RPS6KB1, GADD45B)

> Sporadic pNET: Subset with low VHL and high CA9, Glut1, HIF1

Speisky, Clin Cancer Res 2012

Schmitt, ERC 2009
Hypoxia: 25% sporadic pNET

VHL

HIF-1α

CA-9

GLUT-1

VEGF
Pseudohypoxia: sporadic pNET

Couvelard et al. BJC 2005

Schmitt et al. ERC 2009

CA-9 negative
CA-9 positive

p < 0.0001
(Pseudo)hypoxia sporadic pNET: But!!

Couvelard et al, Br. J. Cancer, 2005
Pseudohypoxia in si-NET

Description of Hif 2α mutation in Somatostatinoma

Somatic HIF2A Gain-of-Function Mutations in Paraganglioma with Polycythemia

Zhengping Zhuang, M.D., Ph.D., Chunzhang Yang, Ph.D., Felipe Lorenzo, M.D., Ph.D., Maria Merino, M.D., Tito Fojo, M.D., Ph.D., Electron Kebebew, M.D., Vera Popovic, M.D., Ph.D., Constantine A. Stratakis, M.D., D.Sc., Josef T. Prchal, M.D., and Karel Pacak, M.D., Ph.D., D.Sc.

Hypoxia in liver metastases

Zhuang et al, NEJM, 2012

Arvidsson et al, ERC, 2010
Conclusion (Pseudo)hypoxia

> Is observed and plays a role in many NET

> But:
> Pseudohypoxia: Different genes involved in NET of different organs
> Phenotype of Hypoxia: can be chicken or egg?
> Is this different for treatment?
PCC, MTC, pNET (familial syndromes)

> Hypoxia (VHL, SDHx, Hif 2a, PHD3)
  — PCC/PGL, pNET, siNET

> mTOR activation (NF1, TSC2, PTEN)

> Epigenetic changes, chromosomal instability (Men1, DAXX, ATRX)
mTOR activation in pNET (NF1, TSC2, PTEN)

> Germline and somatic mutations
> Response to Everolimus
> No prediction yet
> Less effective in si-NET/lung
Hyperactivated kinase signalling: Sunitinib treatment of pNET

> Multi tyrosin kinase inhibitor
> Possible targets:

Raymond et al, NEJM 2012

Faivre et al. 2007, Nature Reviews Drug Discovery
Lung-NET, pNET (familial syndromes)

> Hypoxia (VHL, SDHx, Hif 2a, PHD3)
  — PCC/PGL, pNET, siNET

> mTOR activation (NF1, TSC2, PTEN)

> Epigenetic changes, chromosomal instability (Men1)
DAXX/ATRX are frequently mutated in sporadic pNETs

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\begin{align*}
MEN1 & \quad (20\%) \\
DAXX/ATRX & \quad (40\%)
\end{align*}
\]

Chromatin assembly and histone remodelling

Jiao Y. et al. Science 2011
DAXX/ATRX expression in human pNETs and ALT correlation

Marinoni I. et al. Gastroenterology 2014
DAXX/ATRX loss and ALT activation predict for bad outcome

Chromatin assembly and histone remodelling

Marinoni I. et al. Gastroenterology 2014
Epigenetics in other NET

- ALT seems to be specific for pNET
- Mutations in these epigenetic modifiers absent in si-NET
- Pulmonary NET have mutations in epigenetic modifiers (MEN1, PSIP1 and ARID1A), most frequently in histone modifiers
Summary

> Hypoxia (VHL, SDHx, Hif 2a, PHD3)
  — PCC/PGL, pNET, siNET

> mTOR activation (NF1, TSC2, PTEN)

> Epigenetic changes, Histone modifications, (pNET and Lung NET)
Possibilities
Needs

- Standardization
- Interdisciplinary collaboration
- Clinical trials
  - Follow-up
- Networks
- Tissue banks