Endocr Pathol (2016) 27:200–204
DOI 10.1007/s12022-016-9447-2

New Genetics and Genomic Data on Pancreatic Neuroendocrine Tumors: Implications for Diagnosis, Treatment, and Targeted Therapies

Anja M. Schmitt[1] · **Ilaria Marinoni**[1] · **Annika Blank**[1] · **Aurel Perren**[1]

Published online: 25 July 2016

Abstract The recent findings on the roles of death-associated protein 6/α-thalassemia/mental retardation X-linked (DAXX/ATRX) in the development of pancreatic neuroendocrine tumors (PanNETs) have led to major advances in the molecular understanding of these rare tumors and open up completely new therapeutic windows. This overview aims at giving a simplified view on these findings and their possible therapeutic implications. The importance of epigenetic changes in PanNET is also underlined by recent findings of a cross-species study on microRNA (miRNA) and messenger RNA (mRNA) profiles in PanNETs.

Keywords Review · Pancreatic neuroendocrine tumor · DAXX/ATRX · Epigenetics · Methylation · Alternative lengthening of telomeres

Introduction

Pancreatic neuroendocrine tumors (PanNETs) harbor a significant malignant potential with 50 % of patients dying of their tumor within 10 years [1]. Although the options for medical therapies have increased during the last decade, as yet there is no tailored therapy for individual patients. This is on the one hand due to the fact that PanNETs are genetically heterogenous and do not share many classical pathways of tumorigenesis with their non-endocrine counterparts [2]. On the other hand, although targeted therapies with the mTOR inhibitor everolimus and the tyrosine kinase inhibitor sunitinib have been approved for the treatment of PanNETs since 2011, due to the lack of systemic collaboration between pathologists, oncologists, and pharmaceutical industries, it is still not known which subset of patients will respond to these compounds. The recent findings on the roles of DAXX/ATRX in the development of PanNETs have led to major advances in the molecular understanding of these rare tumors and open up completely new therapeutic windows. This overview aims at giving a simplified view on these findings and their possible therapeutic implications. The importance of epigenetic changes in PanNET is also underlined by recent findings of a cross-species study on microRNA (miRNA) and messenger RNA (mRNA) profiles in PanNETs.

✉ Anja M. Schmitt
anja.schmitt@pathology.unibe.ch

[1] Institute of Pathology, University of Bern, Murtenstrasse 31, 3010 Bern, Switzerland

DAXX and ATRX Mutations

The first whole exome sequencing analysis on 68 unselected PanNETs by Jiao et al. confirmed mutations of mTOR pathway genes in 15 % of PanNET, while the MEN1 gene was shown to be mutated in 20 % of them. Importantly, in 40 % of the tumors, the authors found mutations in either one of two "new" genes, *DAXX* (death-associated protein 6, located at 6p21.3) and *ATRX* (α-thalassemia/mental retardation X-linked, located at Xq13.1-q21.1) [2]. *DAXX* and *ATRX* mutations are mutually exclusive and are associated with loss of the respective proteins [2, 3]. Clinically, *DAXX/ATRX*-mutated PanNETs are associated with large tumor size and late tumor stage as well as with a significant shortened disease-free and tumor-specific survival [3] in surgical series. Interestingly, DAXX- and ATRX-negative PanNETs are characterized by a telomerase-independent mechanism of telomere length maintenance termed ALT (alternative lengthening of telomeres) [3, 4]. A short overview of the functions of DAXX and ATRX is given in the next section.

DAXX and ATRX Are Involved in Chromatin Remodeling

ATRX is a chromatin remodeler recognizing histone-modification states and modulating chromatin dynamics. It recognizes the N-terminal tail of histone H3 if it is tri-methylated at Lys9 (H3K9) which is a marker of heterochromatin. ATRX binds to histone H3 and forms a complex with its partner DAXX. DAXX, an H3.3-specific histone chaperone, thereupon deposits the histone variant H3.3. Upon H3.3 deposition, DAXX/ATRX complex recruits the histone methyltransferase SUV39H, which methylates the lysine 9 keeping the chromatin in its repressive status. Of note, this process takes place at telomeric and pericentromeric heterochromatic regions of the genome with highly repetitive sequences [5] (Fig. 1). Mutations of either DAXX or ATRX thus may lead to an impairment of the heterochromatin structure and to telomeric dysfunction, probably resulting in ALT. The chromatin remodeling functions of DAXX and ATRX were comprehensively reviewed by Maze et al. [5].

DAXX/ATRX-Deficient PanNETs Are Characterized by ALT

During tumor progression, telomeres first shorten and then become critically short, leading to massive apoptosis. In order for tumor cells to survive and to immortalize, their telomeres must be stabilized either by telomerase or by ALT. ALT is a telomerase-independent means of telomere stabilization which is caused by homologous recombination of telomeric regions. Heaphy et al. demonstrated that loss of either DAXX or ATRX results in an ALT phenotype which is characterized by very faint and tiny and very bright and large telomere signals next to each other in a telomere FISH assay [4] (Fig. 2).

Loss of DAXX/ATRX and ALT Correlates with CIN

Chromosomal instability (CIN) is a hallmark of malignancy. It is known that CIN is a feature of large PanNETs and of PanNET metastases [6, 7]. For many years, the reason for CIN in PanNETs could not be found. Recently, it could be shown that both loss of DAXX/ATRX and the activation of the ALT phenotype in PanNETs correlate significantly with CIN, suggesting that DAXX/ATRX mutations are responsible for CIN in PanNETs [3]. While it is known that very short telomeres, as observed in ALT, can drive CIN [8], the mechanism via which DAXX/ATRX loss would induce CIN is still unclear, and probably changes in the epigenetic status are involved in the process.

Model of PanNET Progression

From the sections above, we have learned that loss of DAXX/ATRX via telomeric dysfunction may contribute to ALT

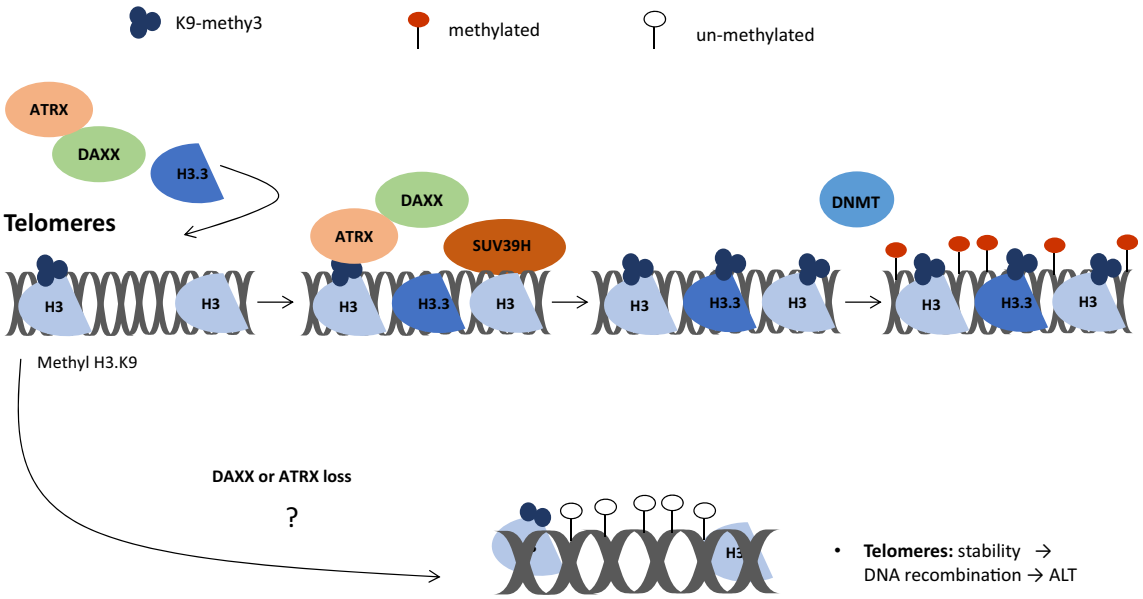

Fig. 1 At telomeric and pericentromeric heterochromatin regions, ATRX recognizes the N-terminal tail of histone H3 if it is tri-methylated at Lys9 (H3K9). ATRX binds to histone H3 and forms a complex with its partner DAXX. DAXX, an H3.3-specific histone chaperone, thereupon deposits the histone variant H3.3. Upon H3.3 deposition, DAXX/ATRX complex recruits the histone methyltransferase SUV39H, which methylates the lysine 9 keeping the chromatin in its repressive status. This process does not take place if DAXX and/or ATRX is lost, finally leading to ALT. *H3* histone H3, *H3.3* histone H3.3, *DNMT* DNA methyltransferase 1, *ALT* alternative lengthening of telomere

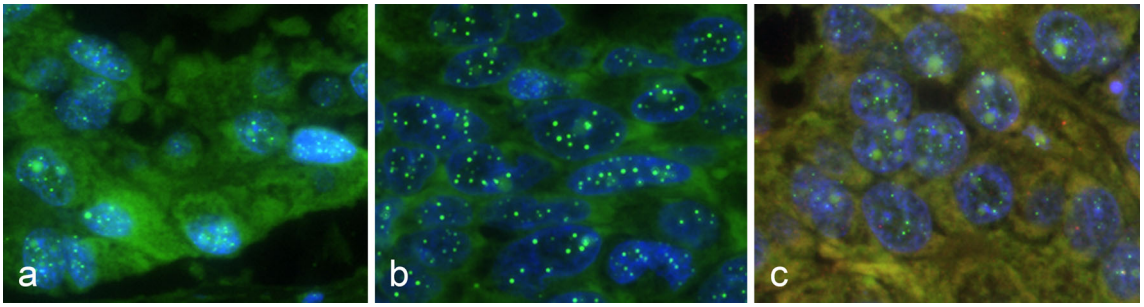

Fig. 2 Telomeric FISH of three different PanNET cases. **a**, **b** Two PanNET cases showing the ALT phenotype with very large and bright signals next to very small and faint signals. **c** In contrast, a PanNET without ALT phenotype shows homogeneously sized signals

activation and to CIN. DAXX/ATRX-deficient PanNETs and PanNETs showing the ALT phenotype as well as CIN correspond to large and late stage tumors. It thus can be hypothesized that *DAXX/ATRX* mutations represent a transforming, i.e., late rather than an initiating/early event in PanNET tumorigenesis. DAXX/ATRX mutations via ALT activation and CIN then lead to clonal heterogeneity, followed by a selection of clones and finally to metastases [3]. This model is supported by the fact, that among DAXX/ATRX-deficient PanNETS <2 cm, 85.8 % of tumors do not show the ALT phenotype, whereas among DAXX/ATRX-deficient PanNETS >2 cm, 95 % of tumors show the ALT phenotype [3]. Interestingly, mice in which DAXX knock-out in the beta cells of the pancreatic islets is induced after tamoxifen induction at 4 weeks of age (DAXX(fl/fl)CreT) do not show any tumor formation. Islets of 70 weeks old Daxx (−/−) mice histologically cannot be differentiated from islets of wild-type mice (Marinoni et al., unpublished data). These findings do not only support the suggested progression model but also show that *DAXX* mutations alone are not sufficient to induce a PanNET but that an initiating event is indispensable for tumor formation. This initiating event in 20 % of the cases is a *MEN1* mutation [2]. From MEN1 patients, it is known that the majority of the pancreatic tumors correspond to microadenomas and do not show any progression [9]. In a recent study on PanNET in MEN1 patients, de Wilde et al. demonstrated that none of the microadenomas and none of the tumors <3 cm investigated showed a loss of DAXX/ATRX whereas in 25 % of the tumors >3cm and in the metastases thereof, a loss of DAXX/ATRX could be shown [10].

DAXX and ATRX Are Involved in DNA Methylation

Besides their function in telomere maintenance via histone H3.3 deposition, DAXX and ATRX play also an important role in DNA methylation. DAXX recruits DNA methyltransferase 1 (Dnmt1, also referred to as “maintenance Dnmt”) to target promoters, and ATRX contains an ADD domain interacting with the same histone marks as DNA methyltransferases 3A and 3L [11]. This is in line with the findings of genome scale DNA methylation profiles suggesting a correlation between DNA methylation and histone methylation patterns [12]. Thus, DAXX and potentially ATRX not only are involved in telomere maintenance but are also important players in DNA methylation. The impact of DAXX and ATRX on DNA methylation was recently investigated by Pipinikas and colleagues in the first genome-wide DNA methylation study of 53 PanNET tumor tissues. The study demonstrates that DAXX- and ATRX-deficient tumors exhibit a reversed DNA methylation pattern in comparison to normal pancreatic tissue. As the authors analyzed DAXX and ATRX separately in their cohort, they found loss of DAXX to have a stronger effect on the change of the DNA methylation pattern than loss of ATRX. Although the authors also investigated DAXX/ATRX-proficient tumors, the authors did not report the effect of DAXX/ATRX deficiency in comparison to DAXX/ATRX proficiency on DNA methylation in their study [13]. Stefanoli and colleagues investigated promoter hypermethylation of 33 tumor suppressor genes and LINE-1 methylation in 56 PanNETs. They found that a subgroup of PanNETs with poor prognosis showed a distinct hypermethylation of tumor suppressor genes while a different subset of PanNETs with poor prognosis demonstrated hypomethylation of LINE-1, which is regarded as a surrogate marker for the global DNA methylation status [14]. The authors do not provide any information about DAXX and ATRX mutations in their cohort, but it can be hypothesized that the described tumor subsets could correspond to DAXX/ATRX-deficient tumors.

Therapeutic Implications of *DAXX/ATRX* Mutations in PanNETs

As ALT does not occur in normal but only in tumor tissues, it would represent an ideal target for tumors with an ALT phenotype. Only recently, Flynn et al. could demonstrate a persistent association of replication protein A (RPA) with telomeres after DNA replication observed in ATRX deficient,

ALT-positive cervical cancer, and osteosarcoma cell lines. Importantly, the authors showed that the inhibition of the protein kinase ATR, which is a crucial regulator of homologous recombination and which is recruited by RPA, is able to disrupt ALT and to induce chromosome fragmentation and apoptosis in ALT cells [15].

Given the involvement of DAXX and ATRX in DNA methylation and histone deposition, DNMT inhibitors as well as histone deacetylase inhibitors (HDIs) represent a further therapeutic option for targeting DAXX/ATRX-deficient PanNETs. Although monotherapies with HDI which are FDA approved for several hematologic malignancies have shown disappointing results in solid tumors, recent trials have demonstrated that the synergistic combination of epigenetic agents with chemotherapy, hormonal therapy, or other epigenetic agents shows a favorable effect on survival [16, 17].

Further studies will elucidate the potential and promising role of the discussed compounds in PanNETs.

miRNA and mRNA Profiles Identify Different Types of PanNETs

The importance of epigenetic mechanisms is supported by a recent cross-species study. Based on distinct miRNA and mRNA profiles, three different types of PanNET were described that correlated with some degree with mutational pattern, tumor grade, metastatic potential, functionality, and metabolic features [18]. Insulinoma-like tumors (ITs) are usually functional tumors with a low proliferation index corresponding either to G1 or G2, a low metastatic potential, and a gene expression pattern similar to normal β cells, including expression of metabolic enzymes of the β cell lineage. In these tumors, mutations in *MEN1*, *mTOR* pathway genes, and *ATM* but not in *DAXX/ATRX* are found. Intermediate or MEN1-like tumors are non-functional tumors with a low to moderate proliferation index corresponding to G1 and G2 and with a moderate metastatic potential. These tumors typically show *MEN1* mutations. The metastasis-like primary (MLP) tumors are non-functional tumors with a high proliferation index corresponding in the majority of cases to G2 and G3 and with a high metastatic potential. In contrast to the IT subtype, they show a gene expression pattern similar to islet precursor/stem cells and epithelial-mesenchymal transition, including metabolic enzymes not expressed in mature β cells. These tumors show mutations in *MEN1*, *DAXX/ATRX*, *mTOR* pathway genes, and *ATM*. The fact that the authors found a strong correlation between mRNA and miRNA and the different PanNET subtypes but only a partial correlation with mutations once again argues for an eminent role of epigenetic changes in the development of PanNETs. Based on the findings of this study, targeting of specific miRNAs or metabolic pathways might be part of a personalized therapy in the future.

Summary

In summary, due to the availability of large-scale genomic data, the understanding of the biologically heterogeneous group of PanNET has made a big step forward. Concerning targeted therapy, it was not possible yet to link mutations to therapy response. Recent findings suggest that epigenetic changes play also an important role. Methylation, histone modification, and ALT represent potential new targets in the tailored therapy of *DAXX/ATRX*-mutated PanNET, while ALT would be an ideal target as it does not occur in normal tissues. Furthermore, targeting of specific miRNAs or metabolic pathways might be part of a personalized therapy in the future. More than ever, there is a strong need for the collaboration of pathologists, oncologists, and pharmaceutical industries to elaborate possible therapeutic strategies in the more and more complex biology of PanNETs.

References

1. Halfdanarson TR, Rubin J, Farnell MB et al. (2008) Pancreatic endocrine neoplasms: epidemiology and prognosis of pancreatic endocrine tumors. Endocr Relat Cancer 15(2):409–427
2. Jiao Y, Shi C, Edil BH et al (2011) DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science 331(6021):1199–1203
3. Marinoni I, Kurrer AS, Vassella E et al. (2014) Loss of DAXX and ATRX are associated with chromosome instability and reduced survival of patients with pancreatic neuroendocrine tumors. Gastroenterology 146(2):453–460 e5
4. Heaphy CM, de Wilde RF, Jiao Y et al. (2011) Altered telomeres in tumors with ATRX and DAXX mutations. Science 333(6041):425
5. Maze I, Noh KM Allis CD (2013) Histone regulation in the CNS: basic principles of epigenetic plasticity. Neuropsychopharmacology 38(1):3–22
6. Jonkers YM, Claessen SM, Perren A et al. (2005) Chromosomal instability predicts metastatic disease in patients with insulinomas. Endocr Relat Cancer 12(2):435–447
7. Zhao J, Moch H, Scheidweiler AF et al. (2001) Genomic imbalances in the progression of endocrine pancreatic tumors. Genes Chromosomes Cancer 32(4):364–372
8. Kong CM, Lee XW Wang X (2013) Telomere shortening in human diseases. FEBS J 280(14):3180–3193
9. Anlauf M, Enosawa T, Henopp T et al. (2008) Primary lymph node gastrinoma or occult duodenal microgastrinoma with lymph node metastases in a MEN1 patient: the need for a systematic search for the primary tumor. Am J Surg Pathol 32(7):1101–1105
10. de Wilde RF, Heaphy CM, Maitra A et al. (2012) Loss of ATRX or DAXX expression and concomitant acquisition of the alternative lengthening of telomeres phenotype are late events in a small subset of MEN-1 syndrome pancreatic neuroendocrine tumors. Mod Pathol 25(7):1033–1039
11. Puto LA Reed JC (2008) Daxx represses RelB target promoters via DNA methyltransferase recruitment and DNA hypermethylation. Genes Dev 22(8):998–1010
12. Meissner A, Mikkelsen TS, Gu H et al. (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454(7205):766–770

13. Pipinikas CP, Dibra H, Karpathakis A et al. (2015) Epigenetic dysregulation and poorer prognosis in DAXX-deficient pancreatic neuroendocrine tumours. Endocr Relat Cancer 22(3):L13–L18
14. Stefanoli M, La Rosa S, Sahnane N et al. (2014) Prognostic relevance of aberrant DNA methylation in g1 and g2 pancreatic neuroendocrine tumors. Neuroendocrinology 100(1): 26–34
15. Flynn RL, Cox KE, Jeitany Met al. (2015) Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. Science 347(6219):273–277
16. Juo YY, Gong XJ, Mishra A et al. (2015) Epigenetic therapy for solid tumors: from bench science to clinical trials. Epigenomics 7(2):215–235
17. Zahnow CA, Topper M, Stone M et al. (2016) Inhibitors of DNA Methylation, Histone Deacetylation, and Histone Demethylation: A Perfect Combination for Cancer Therapy. Adv Cancer Res 130:55–111
18. Sadanandam A, Wullschleger S, Lyssiotis CA et al. (2015) A Cross-Species Analysis in Pancreatic Neuroendocrine Tumors Reveals Molecular Subtypes with Distinctive Clinical, Metastatic, Developmental, and Metabolic Characteristics. Cancer Discov 5(12):1296–1313