REVIEW

DICER1 gene mutations in endocrine tumors

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Abstract

In this review, the importance of the *DICER1* gene in the function of endocrine cells is discussed. There is conclusive evidence that *DICER1* mutations play a crucial role in the development, progression, cell proliferation, therapeutic responsiveness and behavior of several endocrine tumors. We review the literature of *DICER1* gene mutations in thyroid, parathyroid, pituitary, pineal gland, endocrine pancreas, paragangliomas, medullary, adrenocortical, ovarian and testicular tumors. Although significant progress has been made during the last few years, much more work is needed to fully understand the significance of *DICER1* mutations.

Introduction

In the last few years, the *DICER1* gene and its mutations generated interest and have been investigated by several researchers. Results have shown that heterozygous germline *DICER1* mutations cause a familial *DICER1* tumor syndrome. This syndrome is characterized by a number of unusual tumors, both benign and malignant, such as pleuropulmonary blastoma (PPB), ovarian sex cord-stromal tumors, ciliary body medulloepitheliomas, nasal chondromesenchymal hamartomas, multinodular goiter and differentiated thyroid carcinomas, among others (Foulkes *et al.* 2014). Prototypical renal manifestations of *DICER1* mutation are cystic nephroma and, more rarely, anaplastic sarcoma of kidney. *DICER1* mutations have also been documented in pediatric tumors including Wilms tumor and in pituitary blastoma (PitB), as well as

in tumors of various endocrine organs (Hill *et al.* 2009, Huang & Yao 2010, Schultz *et al.* 2011, Heravi-Moussavi *et al.* 2012, Darrat *et al.* 2013, de Kock *et al.* 2014a,b,c, 2017, Foulkes *et al* 2014, Costa *et al.* 2015, de Sousa *et al.* 2015). The rare pediatric PitB appears to be virtually pathognomonic for *DICER1* mutations, which causes tumors in other endocrine organs as well (Fig. 1).

DICER1 is located on chromosome 14q32.13. It is composed of 27 exons and encodes a cytoplasmic class 3 ribonuclease (RNase-III), which plays a crucial role in the biogenesis of microRNAs (miRNAs). Human DICER1 protein, also known by several synonyms (Table 1) contains 1922 amino acids (Fig. 2). It cleaves double-stranded RNA precursors into small-interfering RNA (siRNA) and miRNA, both of which are evolutionarily
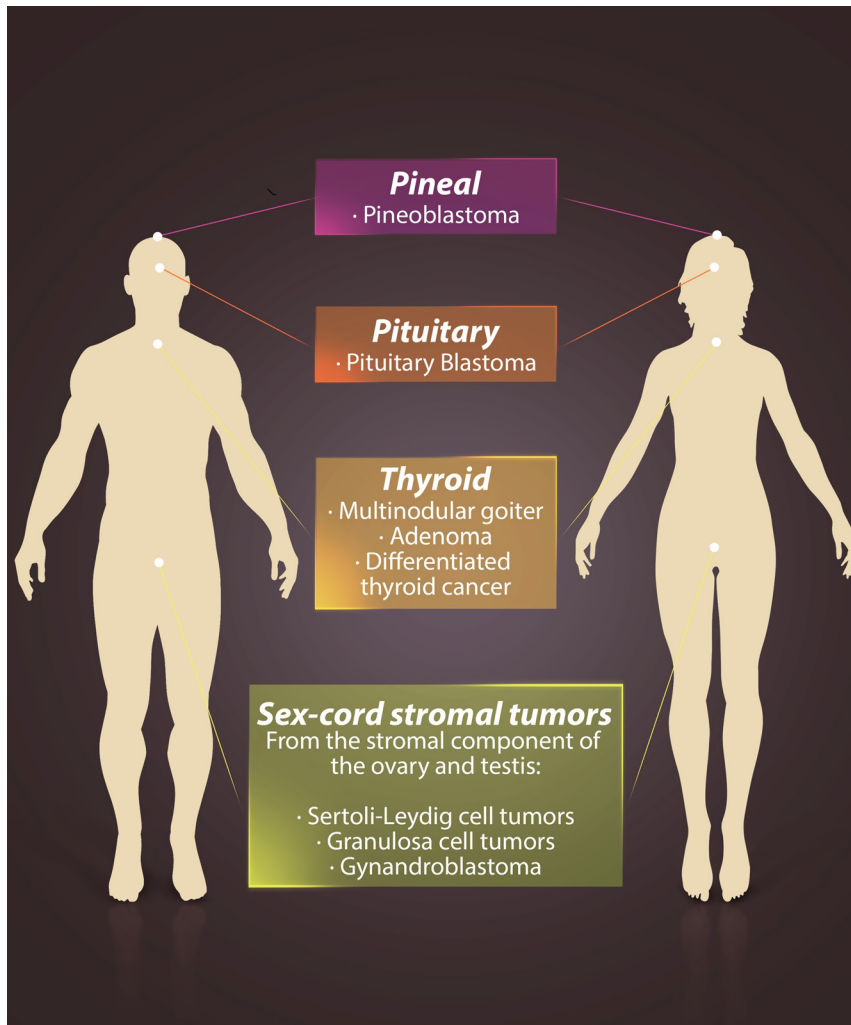
Figure 1
Schematic diagram of the various locations of endocrine tumors associated with *DICER1* syndrome. A full colour version of this figure is available at https://doi.org/10.1530/ERC-17-0509.

conserved, small non-coding RNAs, approximately 21–23 nucleotides in length. siRNAs and miRNAs are incorporated into the RNA-induced silencing complex (RISC) and direct it to complementary RNAs, which cleave complementary target mRNA sequences using RNase. This process, known as RNA interference or gene silencing, prevents translation and thereby inhibits gene expression (Fig. 3) (Bernstein *et al.* 2001, Foulkes *et al.* 2014).

Endocrine tumors arise in hormone-producing cells of the endocrine glands which include the pituitary, thyroid, parathyroid, pineal body, adrenal cortex and medulla, ovaries and testes (Nicholson 2008). Recently, more evidence on the importance of *DICER1* role in tumors is emerging (Lambertz *et al.* 2010, Ravi *et al.* 2012, Foulkes *et al.* 2014). The present paper aims to review the role of *DICER1* gene in endocrine cell tumors. Common bioinformatics resources for *DICER1* syndrome are annotated in tabular form (Table 2).

DICER1 mutations in thyroid tumors

While tumors of the thyroid are the most common amongst the endocrine glands, it is not the most studied in relation to mutations in *DICER1*. Germline mutations in *DICER1* have been found in individuals with multinodular goiter (MNG) (Rio Frio *et al.* 2011, Darrat *et al.* 2013, Rath *et al.* 2014, Rossing *et al.* 2014, Wu *et al.* 2014, Rutter *et al.* 2016). While MNG is not malignant, a few studies suggest that the rate of thyroid cancer may be 10–20% higher in patients with *DICER1* mutations with MNG (Smith *et al.* 2013). A 2011 study (Rio Frio *et al.* 2011) focusing on *DICER1* mutations in familial MNG concluded that *DICER1* mutations were responsible for autosomal dominant familial MNG and MNG with SLCT and that they do not need to coexist with PPB. miRNA analysis of lymphoblastoid cell lines from affected and unaffected familial MNG cases revealed *DICER1* mutation carriers had deviations in their miRNA

Table 1 Synonyms and previous nomenclature for the *DICER1* gene used in the literature.

DICER1
DCR1
Dicer
Dicer 1 ribonuclease III
Dicer 1, double-stranded RNA-specific endoribonuclease
Dicer 1, ribonuclease type III
Dicer1, DCR-1 homolog
DICER1_HUMAN
Endoribonuclease dicer
Helicase MOI
Helicase-moi
Helicase with RNAse motif
HERNA
K12H4.8-LIKE
KIAA0928
MNG1

(Rio Frio *et al.* 2011). Although studies have focused on the possible existence of a correlation between *DICER1* mutations, MNG and thyroid cancer, that link is not clear. Specifically, some differentiated thyroid carcinoma (DTC) may not arise from MNG and the risk of DTC given that an individual has MNG is unknown.

Recent studies have provided more direct links between *DICER1* mutations and thyroid cancer. In a case series of three patients with DTC and PPB, acquired somatic *DICER1* mutations in thyroid tissue, accompanied by inherited germline mutations in *DICER1* were identified (de Kock *et al.* 2014c). The somatic mutations in these individuals were hotspot mutations, interfering with metal ion-coordinating residues in the RNase IIIb domain of *DICER1*, which have also been observed in other individuals with DTC and germline *DICER1* mutations (Rutter *et al.* 2016). Somatic hotspot mutations (E1813G, E1813K, D1810H) have also been observed in papillary thyroid

carcinoma (PTC) without germline *DICER1* mutation and are predicted to have a high impact on the function of the DICER1 protein (Agrawal *et al.* 2014, Costa *et al.* 2015, Landa *et al.* 2016). In a study of sporadic follicular thyroid cancers, two minimally invasive follicular thyroid carcinomas (miFTC) and two benign follicular adenomas (FA) were found to carry somatic mutations in *DICER1* (Yoo *et al.* 2016). These mutations (E1705Q, D1810H, E1813G and E1813Q) were also found to be in the RNase III domain. DICER1 mRNA downregulation and altered expression of miRNAs have been observed in benign and malignant thyroid samples, including follicular adenomas and PTC as well (Erler *et al.* 2014).

The risk of DTC is modestly elevated with the existence of *DICER1* mutations but in a 2012 publication, they reported the development of follicular thyroid carcinoma after the patients were treated for PPB using both high-dose chemotherapy (HDC) and hematopoietic stem cell transplantation (HSCT) (Shin *et al.* 2012). Although literature is limited, they found that there was a particularly high prevalence rate (60%) of follicular thyroid carcinomas in patients with PPB with a *DICER1* mutation, who were treated with HDC and HSCT. It is known that genetic anomalies, as well as, chemotherapies and radiotherapies contribute to the development of secondary thyroid carcinomas, but this study found that using HDC and/or HSCT treatments can increase the susceptibility for patients with PPB to develop secondary follicular thyroid carcinoma (Cohen *et al.* 2007, Taylor *et al.* 2009, Shin *et al.* 2012).

To date, the role of *DICER1* mutations in thyroid cancer remains understudied and warrants further analysis, especially given the prevalence of thyroid cancers in the population.
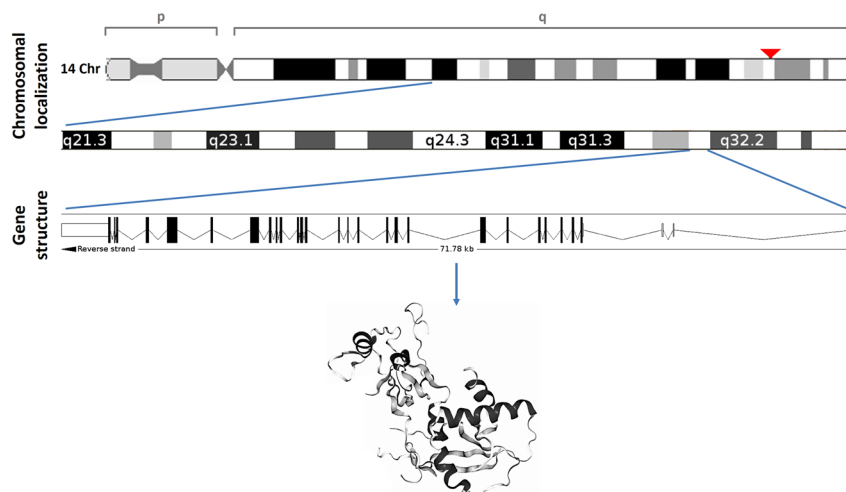
Figure 2
Schematic diagram of the role of *DICER1* in miRNA processing. The function of the *DICER1* gene is to cleave double stranded RNA precursors into small interfering RNA (siRNA) and miRNA both of which are evolutionarily conserved, small non-coding RNAs approximately 21–23 nucleotides in length. A full colour version of this figure is available at https://doi.org/10.1530/ERC-17-0509.
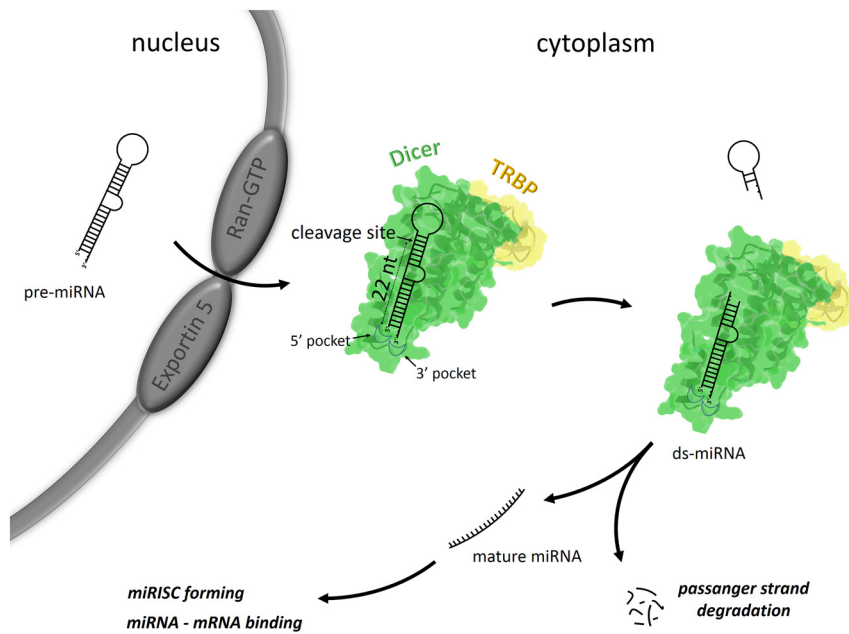
Figure 3

The cytogenetic location of the *DICER1* gene is on the long arm of chromosome 14 at position 32.13. A full colour version of this figure is available at https://doi.org/10.1530/ERC-17-0509.

DICER1 mutations in ovarian and testicular cancer

An extensive amount of research revealed the relationship between *DICER1* mutations and ovarian tumors of non-epithelial and stroma-cell origin (de Kock *et al.* 2017). There has been considerably less work performed to examine this relationship in gonadal tumors of endocrine origin (Kim *et al.* 2013, Schultz *et al.* 2014). This is likely due to the fact that these types of tumors represent a small fraction of gonadal cancers (Winter & Albers 2011, Van Nieuwenhuysen *et al.* 2013). Additionally, to our knowledge, all research performed in this area has focused on ovarian tumors, with little to no work performed on tumors of testicular origin.

Research on *DICER1* in endocrine gonadal tumors began with the observation that there were an unusually high number of individuals with ovarian sex-cord stromal tumors (OSCST) and familial cases of other lesions associated with *DICER1* syndrome. Schultz and coworkers identified several cases of OSCST in individuals with familial PPB, which is known to be associated with *DICER1* germline mutations (Hill *et al.* 2009, Schultz *et al.* 2011). They identified four *DICER1* mutations in six patients with OSCST with family history of PPB and two mutations in patients without PPB kindreds. These *DICER1* mutations were observed in three different types of OSCST: Sertoli-Leydig cell tumors (SLCT), juvenile granulosa cell tumors and gynandroblastoma. It was also observed that some families with cases of MNG also contained individuals with SLCT (Rio Frio *et al.* 2011,

Darrat *et al.* 2013, Wu *et al.* 2014). Germline *DICER1* mutations were observed in both familial MNG and MNG with SLCT studied. It was also shown that miRNA profiling RNA samples from lymphoblastoid cell lines from both affected and unaffected members of familial MNG cases revealed miRNA perturbations in those individuals (Rio Frio *et al.* 2011, Kim *et al.* 2013, Schultz *et al.* 2014, Wu *et al.* 2014). A study by Kim and coworkers demonstrated how OSCSTS harbored *DICER1* mutations that were rarely found in other tumors (Kim *et al.* 2013).

Further research examined the effects of *DICER1* mutations in non-epithelial ovarian cancers and identified so-called 'hotspot' mutations in the *DICER1* RNase IIIb domain in 29% of non-epithelial ovarian tumors (Heravi-Moussavi *et al.* 2012). These mutations were most frequently encountered in Sertoli-Leydig cell tumors (60%) and exclusively affected RNase IIIb function without impairing RNase IIIa function. In their study, they also screened testicular germ-cell tumors to determine whether they also had the same mutations. They found that only one testicular germ-cell tumor harbored the same hotspot mutations noted in the non-epithelial ovarian tumor types screened (Heravi-Moussavi *et al.* 2012). Hotspot mutations affected the D1709 residue in codons encoding metal-ion (Mg^{2+}/Mn^{2+}) binding sites, which aid in the interaction and cleavage of miRNA. Notably, DICER1 function was not completely lost in these tumors, but was altered in specific cell types. An absence of LOH argues against the role of *DICER1* as a two-hit tumor suppressor and suggests it acts more like a dominantly acting oncogene (Heravi-Moussavi *et al.*

Table 2 Bioinformatic resources for *DICER1* syndrome.

Gene and genome	
Ensemble	http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000100697;r=14:95086228-95158010;t=ENST00000343455
NCBI gene	https://www.ncbi.nlm.nih.gov/gene/23405
HGNC	https://www.genenames.org/cgi-bin/gene_symbol_report?hgnc_id=HGNC:17098
GeneCards	http://www.genecards.org/cgi-bin/carddisp.pl?gene=DICER1
Protein	
UniProt	http://www.uniprot.org/uniprot/Q9UPY3
STRING	https://string-db.org/cgi/network.pl?taskId=z7cZyhER4Ky5
Human protein atlas	https://www.proteinatlas.org/search/DICER1
HPRD	http://hprd.org/summary?hprd_id=05875&isoform_id=05875_1&isoform_name=Isoform_1
Clinical resources	
OMIM	https://www.omim.org/entry/606241?search=dicer1&highlight=dicer1
GeneReviews	http://www.ncbi.nlm.nih.gov/books/NBK196157/

HGNC, HUGO Gene Nomenclature Committee; HPRG, Human Protein Reference Database; NCBI, National Center for Biotechnology Information; OMIM, Online Mendelian Inheritance in Man; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; UniProt, Universal Protein Resource.

2012). At present, it is debatable as to the exact mechanism of *DICER1* function. In certain tumors, the LOH may be a result of the presence of a germline mutation in *DICER1*, which is accompanied by a somatic mutation in RNase IIIb domain or it may result in an autosomal dominant pattern whereby only one copy of the altered gene is sufficient to cause the increased risk of developing the tumor. The finding of hotspot mutations in SLCT was confirmed by Kim and coworkers, albeit at a lower rate (15.4%) (Kim *et al.* 2013). These mutations were absent in all other non-epithelial ovarian tumors examined, including sex cord-stromal tumors and granulosa cell tumors. A similar study found *DICER1* somatic mutations in 32% of sex cord-stromal tumors and 3% of germ cell tumors (Witkowski *et al.* 2013). These mutations only affected five residues (E1705, D1709, E1788, D1810 and E1813) all within the RNase IIIb domain. SLCT were the most common tumors affected by these mutations (53%). Goulvent and coworkers observed mutations in the RNase IIIb domain in 31.5% of SLCTs, 25% of juvenile granulosa cell tumors and 8.3% of undifferentiated SCSTs (Goulvent *et al.* 2016). Although this study comprised a small sample (156 SCLTs), they found that more frequent expression of the estrogen receptor and lower age at diagnosis were observed in *DICER1*-mutated tumors, suggesting that *DICER1*-mutated tumors may have a worse prognosis than *DICER1* wild-type tumors.

Analysis of global miRNA and gene expression in SLCTs with *DICER1* hotspot mutations revealed a global reduction of 5p-derived miRNAs due to loss of catalytic activity of the RNase IIIb domain (Wang *et al.* 2015). This deficit in miRNA production was associated with deregulation of genes controlling cell proliferation (ARID3A, CDC25A and PLAGL1) and cell differentiation

(FST, CYP19A1, FGF9 and FGFR2). Silencing of the expression of the let-7 family of miRNA, which have important roles in cell proliferation and differentiation, was proposed to contribute to the tumorigenesis of SLCTs. It is important to note that SLCTs are rare SCSTs that account for 0.5% of all ovarian neoplasms. They are the most virilizing tumor of the ovary and approximately 50% have been shown to be endocrine-inactive tumors, but no data are available to show what percentage of *DICER1* mutation-positive cases are functioning (Gheorghisan-Galateanu *et al.* 2003).

The histologic features of OSCSTs with *DICER1* mutations have not been studied extensively, but appear to have some distinctive features including Sertoli cell and juvenile granulosa cell-like elements (Oost *et al.* 2015). However, even with identical mutations, tumor morphology and immunohistochemical properties can vary drastically, suggesting that other factors are involved. More extensive studies are required to fully understand how *DICER1* mutations contribute to the histologic features of OSCSTs.

Current research on *DICER1* in gonadal tumors is aimed at not only finding new therapeutic approaches, but also aiding in the classification of problematic or previously unclassifiable OSCSTs, in particular, prognostic information, as that is a key evidence in any cancer consultation (Stewart *et al.* 2013, Witkowski *et al.* 2013). The absence of *DICER1* mutations in unclassified SCSTs may suggest the existence of distinct tumor subtypes with different mechanisms of pathogenesis (Stewart *et al.* 2013). Wang and coworkers found that SLCTs with *DICER1* hotspot mutations frequently presented with various levels of retiform foci, which are typically seen in moderately and poorly differentiated SLCTs (Wang *et al.* 2015).

In addition, SLCTs with hotspot mutations have distinct gene expression landscapes and may represent distinct diseases with different patient outcomes. Improvement of diagnosis accuracy and reclassification of SLCTs may occur in the future, as the genomic alterations in these types of tumors are better understood.

DICER1 mutations in pituitary tumors

Neoplasms of the pituitary gland are not uncommon in the general population. Pituitary adenomas have an incidence of up to 15% of all intracranial tumors (Osamura *et al*. 2017). First described in 2008, pituitary blastomas (PitB), on the other hand, are exceedingly rare embryonal tumors of the pituitary gland, which typically occur in very young children (Scheithauer *et al*. 2008, 2012).

To date, the only pituitary lesion reported to be associated with *DICER1* mutations is PitB. In 2012, Scheithauer and coworkers described a case of a 9-month infant with PitB and a germline heterozygous *DICER1* mutation (Scheithauer *et al*. 2012). A subsequent study examined germline *DICER1* mutations in nine additional children with PitB (de Kock *et al*. 2014b) – germline DNA from 10 children with PitB was analyzed, nine of which harbored germline *DICER1* mutations, with one affecting the RNase IIIb catalytic site, a domain critical to its function. PitB tumor DNA analyzed from these 10 patients demonstrated that of nine that had *DICER1* mutations, eight were somatic. Seven of these mutations were predicted to affect the RNase IIIb catalytic site. The other mutations were loss of heterozygosity (LOH) of the wild-type allele (de Kock *et al*. 2014b).

Using tumor tissue from a 12-month-old female with an aggressive PitB and resultant Cushing's disease, Sahakitrungruang and coworkers performed whole-exome sequencing (WES) and Sanger sequencing of *DICER1* from genomic DNA (Sahakitrungruang *et al*. 2014). This study identified two novel *DICER1* mutations in the DNA of the PitB: a heterozygous germline c.3046delA → (p.S1016VfsX1065) frameshift mutation and a heterozygous somatic c.5438A3T → (p.E1813V) missense mutation. The p.E1813V mutation was located in RNase IIIb catalytic domain. Given that two mutations exist in the tumor DNA, one germline and the other somatic, these findings suggest that loss of function of both *DICER1* alleles is required for tumor development.

A striking characteristic amongst patients with PitB is that they present with features of Cushing disease, with elevated levels of blood ACTH and hypercortisolism. It was also noted that apart from the characteristic features found in many of the PitB cases, they can also coexist with PPB and cystic nephroma (Scheithauer *et al*. 2012, de Kock *et al*. 2014b, Rotondo *et al*. 2017).

Obviously, more work must be performed to understand the role of *DICER1* mutations in PitB. However, the current evidence suggests that germline *DICER1* mutations are a major contributor to the development of PitB, along with a second acquired somatic mutation in the RNase IIIb domain. Such a 'two-hit' model of tumor development is the hallmark of many types of human cancers and appears to be characteristic of DICER1 mutations in PitB.

DICER1 mutations in pineal gland tumors

Several investigations have been done into the role of *DICER1* mutations in the pineal gland. Current research suggests that *DICER1* mutations may contribute to the development of pineoblastoma (PinB), a malignant, primitive neuroectodermal tumor of the pineal gland, typically occurring in children.

In one patient with a malignant PinB, a mutation in exon 8 of the *DICER1*, c.1128_1132delAGTAA, was identified in genomic DNA isolated from the tumor (Sabbaghian *et al*. 2012). This mutation was expected to cause p.Lys376Asnfs*11, a deleterious change to the protein and was present in the tumor in the hemizygous state, but was heterozygous in the germline, suggesting that there was LOH at the *DICER1* locus in the tumor (Sabbaghian *et al*. 2012).

A more recent study by de Kock and coworkers extended the previous finding of the presence of *DICER1* mutations in patients with PinB (de Kock *et al*. 2014a). In 21 cases, six deleterious *DICER1* mutations were identified. Three of these mutations (c.4754C>g, p.(Ser1585*), c.5103C>A, p.(Tyr1701*), c.4633dupT, p.(Ser1545Phefs*7)) were germline in origin and were predicted to prematurely truncate the protein. Two of these mutations were accompanied by LOH of the wild-type allele. Another case demonstrated two nonsense mutations, c.3280_3281delTT (p.(leu1094Argfs*9)) and c.3675C>g (p.(Tyr1225*)), which could not be identified as somatic or germline but were also predicted to prematurely truncate the protein. Another germline mutation, c.1498A>T (p.(Lys500*)), induced a premature stop codon in the helicase domain-encoding sequence of the gene and was accompanied by LOH of the wild-type allele. Somatic analysis was not available for the last two mutations, but germline analysis revealed a c.4050+1g>A germline *DICER1* variant in one case and

a (c.4407_4410delTTCT, p.(Ser1470leufs*19)) mutation in the other. None of the mutations encountered in this study affected the *DICER1* RNase IIIb domain. Based on their findings, the authors suggest that *DICER1* is an important susceptibility gene for PinB and that the means by which the second allele is inactivated may be different from other *DICER1*-related tumors (de Kock *et al.* 2014*a*).

In a retrospective study examining patients with pineal parenchymal tumors (PPTs), seven cases of PinB were studied (Raleigh *et al.* 2017). In one pediatric patient, two inactivating mutations in *DICER1* were identified (p.V1080fs, p.E1813D). These mutations were found in the genomic DNA of the tumor, but were not present in DNA isolated from the patient's blood, suggesting they were somatic in nature. The authors suggest that, in addition to *DICER1* tumor predisposition syndrome, *DICER1* inactivation and miRNA deregulation may contribute to the pathogenesis of sporadic PinB (Raleigh *et al.* 2017).

Similar to PitB, the current research on *DICER1* mutations in PinB seems to suggest a 'two-hit' model of tumorigenesis. As suggested by de Kock and coworkers, however, the means by which the second allele is inactivated may be different from other *DICER1*-related tumors (de Kock *et al.* 2014*a*). The mechanism involved in the inactivation of the second allele in PinB involves an inactivating germline mutation and a subsequent somatic hot-spot mutation in certain nucleotides affecting the RNase IIIb domain (de Kock *et al.* 2014*a*). It is important to note, however, that a germline mutation is not necessary for tumor production, since PinB can arise because of a somatic mutation which leads to inactivation of DICER1 and dysfunction/regulation of miRNA (Raleigh *et al.* 2017). Of course, with only three studies conducted on this topic so far, there is much to be learned about how inactivation of the *DICER1* and miRNA dysfunction leads to tumor growth in the pineal gland.

DICER1 mutations in cancer of the endocrine pancreas

A number of publications have examined the role of *DICER1* in the developing and mature pancreas, as well as the relationship between *DICER1* gene mutations and diabetes. It is evident from these studies that *DICER1* plays a crucial role in the development and maintenance of the adult pancreas (Lynn *et al.* 2007, Morita *et al.* 2009, Tattikota *et al.* 2011, Kanji *et al.* 2013, Wang *et al.* 2014, Özcan *et al.* 2015). The regulatory activity by DICER is crucial for the preservation of normal acinar

cell differentiation and that homozygous *DICER* deletion leads to the loss of acinar cell polarity, which initiates both epithelial-to-mesenchymal transitions and acinar-to-ductal metaplasia (Wang *et al.* 2014). Additionally, it has been demonstrated that interference with *DICER1*/miRNA signaling may lead to defects in insulin signaling and glucose metabolism, perhaps identifying a novel therapeutic target for diabetes mellitus (Kalis *et al.* 2011, Melkman-Zehavi *et al.* 2011, Locke & Harries 2012, Mandelbaum *et al.* 2012, Abuhatzira *et al.* 2015).

Few studies have examined the role of *DICER1* in endocrine pancreatic cancer and the exocrine pancreas (Morris *et al.* 2014). In mice, homozygous knockout of *Dicer1* was demonstrated to initiate epithelial-to-mesenchymal transition (EMT) and acinar-to-ductal metaplasia (ADM) in pancreatic acinar cells (Wang *et al.* 2014). Simultaneous activation of oncogenic Kras, a small GTPase known to promote pancreatic neoplasia (Eser *et al.* 2014), with heterozygous knockout of the *Dicer1* accelerated the initiation of pancreatic intraepithelial neoplasms (PanIN) (Wang *et al.* 2014). Conversely, Kras activation combined with homozygous knockout of *Dicer1* did not affect the rate of PanIN initiation but caused accelerated ADM formation (Wang *et al.* 2014). In contrast, they also found that in the absence of Kras activation following widespread *Dicer1* deletion, acinar cells were permitted to regenerate. Although this was a significant finding, it cannot be excluded that other factors such as a transdifferentiated and/or a progenitor cell population may have contributed to this regeneration of cells within the pancreas (Criscimanna *et al.* 2011, Wang *et al.* 2014) These findings reveal that tumor growth in the exocrine pancreas may be sensitive to DICER1 dosage. Since few studies have focused in this area, further work is necessary to eliminate any potential technical limitations (difficulty attaining complete loss of *Dicer* or the fact that *Dicer1*^fl/fl animals retain one functional *Dicer1* allele) (Wang *et al.* 2014), in order to confirm the role of *DICER1* in pancreatic cancer. In a number of pancreatic cancer cell lines (BxPC-1, Capan-2, Panc-1), Wang and coworkers systematically knocked down DICER1 expression and observed the effects on oncogene and tumor suppressor gene expression (Wang *et al.* 2012). Variable expression profiles of the Kras oncogene and the p53 and PTEN tumor suppressors were observed in the three cell lines when *Dicer1* was knocked out. *Dicer1* knockdown inhibited BxPC-1 tumor growth but promoted Panc-1 tumor growth while having no effect on Capan-1 tumor growth. Clearly, there are different molecular mechanisms involved in the pathogenesis of various pancreatic cancer subtypes,

highlighting the necessity for individualized therapies (Wang *et al.* 2012).

Whether or not these findings will translate to cancers of the endocrine pancreas is yet to be seen. While further investigation may be thwarted by the scarcity of pancreatic cancers of endocrine origin, it is clear that more work must be performed in order to determine if the *DICER1* is a viable therapeutic target.

DICER1 mutations in medullary and adrenocortical tumors

In the last decade, genomic approaches have been successfully applied to adrenocortical and medullary tumors. Exome sequencing identified new major drivers in all tumor types (Espiard *et al.* 2014, Faillot & Assie 2016). Although a substantial amount of research has been conducted in order to determine the role of *DICER1* in the adrenal gland, to our knowledge, there have been no studies identifying *DICER1* mutations in tumors of the adrenal medulla. Studies have mainly focused on the role in the development, maintenance and function of the adrenal medulla and adrenal cortex (Huang & Yao 2010, Huang *et al.* 2012, Krill *et al.* 2013, Robertson *et al.* 2013).

Only one study, to our knowledge, has examined *DICER1* mutations in adrenocortical cancer. de Sousa and coworkers examined a large cohort of adult adrenocortical tumors (ACT) and found no hotspot mutations in 31 adrenal adenomas and 30 adrenal carcinomas (de Sousa *et al.* 2015). Whether or not there were other mutations present is unknown, since only the RNase IIIb domain was sequenced. These authors, however, did find that *DICER1* gene and expression was higher in 51% of adrenocortical carcinomas than in the adenomas studied. When the authors studied the data of the adrenocortical carcinomas alone, they found that a weak *DICER1* expression was correlated with reduced disease-free and overall survival time, larger tumor size, more advanced staging, higher Weiss score and was a predictor of recurrence in adrenocortical carcinomas (ACC) (de Sousa *et al.* 2015). In a similar study, Caramuta and coworkers demonstrated that the DICER1 was overexpressed in adrenocortical carcinomas compared to adrenocortical adenomas (Caramuta *et al.* 2013). The reason for this discrepancy is unclear and needs further research.

A 2013 study by Caramuta and coworkers investigated the clinical and functional impact of *TARBP2* overexpression in adrenocortical carcinomas since it was found that mutations both truncating and framshift in *TARBP2*, a gene which encodes transactivation response (TAR) RNA-binding protein (TRBP) protein, causes diminished TRBP protein expression and a defect in miRNA processing (Melo *et al.* 2009, Caramuta *et al.* 2013). They found that in adrenocortical carcinomas, TRBP was overexpressed and that expression of the *TARBP2* gene was a useful marker to differentiate between adrenocortical adenomas and carcinomas (Caramuta *et al.* 2013). TRBP interacts directly with the DICER1 protein and is essential for the stabilization of the DICER1 protein (Chendrimada *et al.* 2005). The coupling of TRBP with DICER1 is essential for the recruitment of the argonaute 2 (Ago2), an enzyme required by RISC, which targets mRNA to prevent translation (Chendrimada *et al.* 2005).

The lack of research on *DICER1* mutations in adrenocortical cancer likely reflects the fact that adrenocortical cancer has an annual incidence of only 0.5–2 cases per million population (Terzolo *et al.* 2014). With the rarity of ACC and the relatively recent surge in interest of *DICER1*, it is no surprise that few papers have been published on this topic. However, the presence of *DICER1* mutations in many endocrine cancers calls for more research on *DICER1* mutations in the adrenal gland, given the devastating nature of the disease and lack of efficacious treatments (Terzolo *et al.* 2014). Whether or not *DICER1* mutations are present in adrenocortical cancer remains to be seen, but they could present a much-needed therapeutic target for this disease.

DICER1 mutations in paragangliomas

Similar to other endocrine tumors, paragangliomas are uncommon neoplasms, with an annual incidence of 1 in 1.3 million, and metastases has been documented as occurring in only 1–3% of cases (Zak & Lawson 1982, Mertens *et al.* 1993, Trombetta *et al.* 2008). Considerable progress in the understanding of the genetic basis of paragangliomas has been made and many familial forms have been recognized (Toledo & Dahia 2015).

To date, and to our knowledge, there have been no studies undertaken to examine the role of *DICER1* mutations in paragangliomas, likely due to the low prevalence of paragangliomas in the population. However, other genes have been implicated in the pathogenesis of paraganglioma (Kirmani & Young 2008, Lloyd 2011). The discovery of these mutations has led to a better understanding of the pathogenesis of paragangliomas and their prevalence in families. Whether or not a similar discovery will be made with *DICER1* mutations is unknown. Unfortunately, this group did not investigate *DICER1* mutations in this endocrine tumor type.

Literature on paragangliomas has focused on describing and characterizing this particular tumor type and no research group, to our knowledge, has looked at whether *DICER1* mutations plays a role in the development and/or progression of paragangliomas in humans. It is therefore also worthy of future research given the presence of these mutations in other endocrine tumors.

DICER1 mutations in parathyroid tumors

While the role of wild-type *DICER1* has been shown to be necessary for the normal function of the parathyroid in response to hypocalcemia and uremia (Shilo *et al.* 2015), there have been no studies examining the role of *DICER1* mutations in the pathogenesis of parathyroid tumors despite the fact that parathyroid tumors are quite prevalent and many are asymptomatic or have symptoms that are not very obvious. Due to incorrect diagnosis, the morbidity of parathyroid carcinomas remains high although they only account for 0.55% of all parathyroid tumors diagnosed (Schulte & Talat 2012). Given the fact that *DICER1* mutations are prevalent in many tumors of endocrine origin, its role in parathyroid tumors demands attention. Further research should aim to improve disease identification, leading to earlier diagnosis and, ideally, better prognosis for individuals with these tumors.

Future directions and conclusions

The discovery of *DICER1* mutations and the resulting familial tumor predisposition syndrome has led to a surge in interest surrounding the *DICER1* gene and its involvement in physiological and pathophysiological processes. As evident from a review of recent literature, *DICER1* mutations are particularly prevalent in tumors and cancers of endocrine origin and they should be considered a risk factor for these conditions especially when coupled with clinical suspicions such as concurrence PPB. While this line of research is still in its infancy, much progress has already been made in understanding how *DICER1* and miRNA signalling contributes to the pathogenesis of a variety of endocrine tumors. However, like any line of research in its early stages, there remain many questions to be answered.

First and foremost, a priority in this field should be to characterize *DICER1* mutations in the less common endocrine tumors and those whose etiology is less well understood. Though it is well established that *DICER1* mutations play a role in the development of pituitary blastoma, pineoblastoma, gonadal tumors and thyroid

tumors, current research on *DICER1* mutations in pancreatic, parathyroid, adrenomedullary tumors and paragangliomas is severely lacking or non-existent. If *DICER1* mutations are identified in these tumors, it could lead to the discovery of a novel therapeutic target for them, which have few other viable treatments. Additionally, identification of *DICER1* mutations in these tumors may allow for better classification of lesions that are currently unclassifiable or ambiguous in nature (Stewart *et al.* 2013, Witkowski *et al.* 2013). This will lead to faster and more accurate diagnoses by physicians, ultimately allowing for improved and more personalized patient care.

While the identification of *DICER1* mutations in endocrine tumors is a step in the right direction, future research should seek to determine the method by which the *DICER1* gene is mutated and how this mutation contributes to the diseased state at the cellular/molecular level. There is currently controversy surrounding the mechanism by which DICER1 function is lost, specifically whether a classical two-hit theory applies to this gene. For example, in pituitary blastoma and pineoblastoma, a two-hit model in which *DICER1* acts as a tumor suppressor gene seems evident, while in Sertoli-Leydig cell tumors, *DICER1* appears to act as a dominantly acting oncogene (Heravi-Moussavi *et al.* 2012, de Kock *et al.* 2014*b*, Sahakitrungruang *et al.* 2014). The notion that *DICER1* mutations can have variable effects on different cell types is currently unclear, but is of great importance to understanding how *DICER1* mutations lead to tumor development and to what extent they contribute. The study of *DICER1*-related tumors has, in general, shown an overarching consistent molecular pattern: germline mutations which are scattered throughout the gene domains, followed by highly specific somatic mutations on a few critical RNase IIIb domains. This was clearly demonstrated in the number of PPB and SLCT cases studied yet PitB and perhaps PinB may not follow this molecular pattern. Once this discrepancy has been resolved, future research should focus on the exact signaling pathways and molecular constituents that interact with DICER1 and how they are disrupted by *DICER1* mutations. Given the normal function of DICER1, it is likely that impaired miRNA signaling plays a critical role in tumor development. Whether targeting of the miRNA signaling system is a feasible or useful therapeutic option remains to be seen, but is a promising line of future research.

While the extent to which *DICER1* mutations contribute to the development of endocrine tumors has not yet been fully established, there is no doubt that aberrant DICER1 signalling plays at least a small role in

the pathogenesis of these diseases. Though research on this topic is still in its early stages, the mere presence of *DICER1* mutations in so many endocrine tumors suggests that this is a very promising path for future research. It is uncertain whether a better understanding of these mutations will lead to useful clinical interventions and/or treatments, but their prevalence is too great to be overlooked any longer.

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