

Genetics of neuroendocrine and carcinoid tumours

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Abstract

Neuroendocrine tumours (NETs) originate in tissues that contain cells derived from the embryonic neural crest, neuroectoderm and endoderm. Thus, NETs occur at many sites in the body, although the majority occur within the gastro-entero-pancreatic axis and can be subdivided into those of foregut, midgut and hindgut origin. Amongst these, only those of midgut origin are generally argentaffin positive and secrete serotonin, and hence only these should be referred to as carcinoid tumours. NETs may occur as part of complex familial endocrine cancer syndromes, such as multiple endocrine neoplasia type 1 (MEN1), although the majority occur as non-familial (i.e. sporadic) isolated tumours. Molecular genetic studies have revealed that the development of NETs may involve different genes, each of which may be associated with several different abnormalities that include point mutations, gene deletions, DNA methylation, chromosomal losses and chromosomal gains. Indeed, the foregut, midgut and hindgut NETs develop via different molecular pathways. For example, foregut NETs have frequent deletions and mutations of the *MEN1* gene, whereas midgut NETs have losses of chromosome 18, 11q and 16q and hindgut NETs express transforming growth factor- α and the epidermal growth factor receptor. Furthermore, in lung NETs, a loss of chromosome 3p is the most frequent change and p53 mutations and chromosomal loss of 5q21 are associated with more aggressive tumours and poor survival. In addition, methylation frequencies of *retinoic acid receptor- β* , *E-cadherin* and *RAS-associated domain family* genes increase with the severity of lung NETs. Thus the development and progression of NETs is associated with specific genetic abnormalities that indicate the likely involvement of different molecular pathways.

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Introduction

Neuroendocrine tumours (NETs) occur in tissues that contain cells derived from the embryonic neural crest, neuroectoderm and endoderm (Le Douarin 1988, Vinik 2001, Oberg 2002). Thus, NETs may occur at many sites in the body, although the majority occur within the gastro-entero-pancreatic axis (Table 1). Amongst these, more than 50% are carcinoid tumours, which are traditionally considered to be serotonin (5-hydroxytryptamine) secreting and argentaffin positive, and are sub-classified into foregut, midgut and hindgut tumours (Vinik 2001, Oberg 2002). The foregut tumours include carcinoids of the bronchus, lung, thymus, stomach, first portion of the duodenum and pancreas; the midgut tumours derive from the second portion of the duodenum, the jejunum, ileum, appendix and ascending colon; and the hindgut tumours derive from the transverse colon, the descending colon and rectum. However, it is important to note that the

foregut and hindgut tumours are argentaffin negative and have a low content (or none) of serotonin. In view of this, the term carcinoid tumour is now reserved for only those midgut tumours that are serotonin secreting and the term NETs, together with the tissue of origin, is used for the other tumours (Oberg 1998). Thus, in this definition, the midgut carcinoid tumours represent a subset of NETs. NETs and carcinoid tumours usually occur as non-familial (i.e. sporadic) tumours. However, they may sometimes occur as part of familial syndromes such as multiple endocrine neoplasia type 1 (MEN1) (Oberg 1998) and neurofibromatosis type 1 (NF1) (Griffiths *et al.* 1987), although the occurrence of familial isolated midgut carcinoids is rare and has been reported only five times (Eschbach & Rivaldo 1962, Anderson 1966, Kinova *et al.* 2001, Oliveira *et al.* 2001, Pal *et al.* 2001). The familial occurrence of NETs is consistent with a genetic aetiology for NETs. This review will focus on the molecular genetic basis of NETs and carcinoid tumours, together with

Table 1 NETs and their frequencies

Tumours	Percentage (%)
Carcinoids (all, i.e. foregut, midgut and hindgut NETs)	< 50
Pancreatic islet cell tumours	
Gastrinomas	25
Insulinomas	15
VIPomas	6
Glucagonomas	3
Others	< 1

a brief outline of the models for tumour development. However, it is important to note that NETs represent a heterogeneous group of tumours, and that comparisons between different studies (Debelenko *et al.* 1997a, Dong *et al.* 1997, Onuki *et al.* 1999, Zhao *et al.* 2000, Ullmann *et al.* 2002), which have generally not used a World Health Organisation (Brambilla *et al.* 2001, Younossian *et al.* 2002) classification are therefore difficult.

Models of tumour development

The development of tumours may be associated with mutations or inappropriate expression of specific normal cellular genes, which are referred to as oncogenes (Thakker & Ponder 1988, Thakker 1993, 1994, Brown & Solomon 1997). Two types of oncogenes, referred to as dominant and recessive oncogenes, have been described. An activation of dominant oncogenes leads to transformation of the cells containing them, and examples of this are the chromosomal translocations associated with the occurrence of Burkitt's lymphoma and the activating mutations of the RET proto-oncogene in MEN type 2. In these conditions, the mutations, which lead to activation of the oncogene, are dominant at the cellular level, and therefore only one copy of the mutated gene is required for the phenotypic effect. However, in some inherited neoplasms, which may also arise sporadically, such as retinoblastoma (RB) and MEN1, tumour development is associated with two recessive mutations, which inactivate oncogenes, and these are referred to as recessive oncogenes. In the inherited tumours, the first of the two recessive mutations is inherited via the germ cell line and is present in all the cells. This recessive mutation is not expressed until a second mutation, within a somatic cell, causes loss of the normal dominant allele. The mutations causing the inherited and sporadic tumours are similar but the cell types in which they occur are different. In the inherited tumours, the first mutation occurs in the germ cell, whereas in the sporadic tumours both mutations occur in the somatic cell. Thus, the risk of tumour development in an individual who has not inherited the first germline mutation is much smaller, as both mutational events must coincide in the same somatic cell. In addition, the apparent paradox that the

inherited cancer syndromes are due to recessive mutations but dominantly inherited at the level of the family is explained by the fact that, in individuals who have inherited the first recessive mutation, a loss of a single remaining wild-type (WT) allele is almost certain to occur in at least one of the large number of cells in the target tissue. This cell will be detected because it forms a tumour, and almost all individuals who have inherited the germline mutation will express the disease, even though they inherited a single copy of the recessive gene. This model involving two (or more) mutations in the development of tumours is known as the 'two-hit' or Knudson's hypothesis (Knudson 1971, 1974). The normal function of these recessive oncogenes appears to be in regulating cell growth and differentiation, and these genes have also been referred to as anti-oncogenes, tumour suppressor genes or gatekeeper genes (Vogelstein & Kinzler 1993, Kinzler & Vogelstein 1997). An important feature which has facilitated the investigation of these genetic abnormalities associated with tumour development is that the loss of the remaining allele (i.e. the second hit), which occurs in the same somatic cell and gives rise to the tumour, often involves a large scale loss of chromosomal material. This 'second hit' may be detected by a comparison of the DNA sequence polymorphisms in the leukocytes and tumour obtained from a patient, and observing a loss of heterozygosity (LOH) in the tumour. In addition to LOH, the second hit may involve smaller deletions that may be intragenic or point mutations, or aberrant methylation, which is an epigenetic silencing mechanism (Lengauer *et al.* 1997). Methylation of DNA, which is intrinsically utilised in X-chromosome inactivation and imprinting of parental genes (Mompalmer & Bovenzi 2000, Robertson & Jones 2000, Tycko 2000, Jones & Baylin 2002), involves the transfer of a methyl group, by a DNA methyltransferase, to the cytosine of a cytosine-guanine (CpG) dinucleotide. These CpG dinucleotides are often found as clusters, which are referred to as CpG islands (Bird 1986), and they may encompass the regulatory sequences of genes. Indeed, hypermethylation of such promoter regions of genes has been shown to be involved in silencing recessive oncogenes and subsequent tumour development. For example, the Von Hippel Lindau gene has been shown to be hypermethylated in sporadic clear cell renal carcinomas (Herman *et al.* 1994). Studies of methylation patterns of foregut, midgut and hindgut NETs have revealed that five genes (*p14*, *p16*, *MGMT*, *THBS1* and *RARβ*) are more frequently methylated in these NETs when compared with pancreatic neuroendocrine tumours (PETs), whilst two genes (*ER* and *COX2*) are equally methylated in all types of NETs, PETs and normal tissue (Table 2). Furthermore, dominant and recessive oncogenes have been shown to be involved in the development and progression of NETs (Table 3). The underlying mechanisms altering the functions of these genes may involve point mutations, chromosomal deletions (i.e. losses), or duplications (i.e. gains), amplifications or insertions. Chromosomal

Table 2 Methylation patterns in foregut, midgut and hindgut NETs

Gene	Methylation status ^a	Reference
<i>Cyclin-dependent kinase inhibitor-2A (p16)</i>	Colon > stomach > ileum > PETs	Chan <i>et al.</i> (2003)
<i>Adenomatous polyposis of the colon (APC)</i>	NSCLC > SCLC > AC and TC	Toyooka <i>et al.</i> (2001)
<i>H-cadherin (CDH13)</i>	NSCLC > SCLC > AC and TC	Toyooka <i>et al.</i> (2001)
<i>O⁶-methylguanine-DNA-methyl transferase (MGMT)</i>	Duodenum = rectum > colon > PETs	Chan <i>et al.</i> (2003)
<i>Retinoic acid receptor-β 2 (RARβ)</i>	Stomach = rectum > ileum > normal tissue > PETs	Chan <i>et al.</i> (2003)
<i>E-cadherin (CDH1)</i>	SCLC > AC and TC	Toyooka <i>et al.</i> (2001)
<i>RAS-associated domain family 1A (RASSF1A)</i>	SCLC > AC and TC	Toyooka <i>et al.</i> (2001)
<i>p14 (cyclin-dependent kinase inhibitor-2D)</i>	SCLC > AC and TC > NSCLC	Dammann <i>et al.</i> (2001), Toyooka <i>et al.</i> (2001)
<i>Cyclo-oxygenase 2 (COX2)</i>	Stomach = rectum > ileum > PETs	Chan <i>et al.</i> (2003)
<i>Oestrogen receptor (ER)</i>	Stomach = rectum > colon > ileum > normal tissue = PETs	Chan <i>et al.</i> (2003)
<i>Thrombosporin 1 (THBS1)</i>	Stomach > ileum > PETs > duodenum = colon > normal tissue	Chan <i>et al.</i> (2003)
<i>Caspase 8 (CASP8)</i>	Rectum > duodenum = stomach > colon > ileum > PETs	Chan <i>et al.</i> (2003)
	SCLC > AC and TC > NSCLC	Shivapurkar <i>et al.</i> (2002)

^aNSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; AC, atypical lung carcinoid; TC, typical lung carcinoid.

Table 3 Dominant and recessive oncogenes altered in NETs

	Chromosomal location	Reference
Dominant oncogenes		
<i>K-ras</i>	12p12.1	Couce <i>et al.</i> (1999), Maitra <i>et al.</i> (2000)
<i>N-ras</i>	1p13.2	Arany <i>et al.</i> (1994)
Recessive oncogenes		
<i>Fragile histidine triad (FHIT)</i>	3p14.2	Kovatich <i>et al.</i> (1998), Onuki <i>et al.</i> (1999)
<i>p16</i>	9p21	Onuki <i>et al.</i> (1999)
<i>APC</i>	5p21	Levin <i>et al.</i> (1995), Petersen <i>et al.</i> (1997)
<i>Mutated in colorectal cancer (MCC)</i>	5p21	Levin <i>et al.</i> (1995), Petersen <i>et al.</i> (1997)
<i>MEN1</i>	11q13	Gortz <i>et al.</i> (1999), D'Adda <i>et al.</i> (1999b)
<i>Succinate-ubiquinone oxidoreductase subunit D (SDHD)^a</i>	11q23	Kytola <i>et al.</i> (2002)
<i>Transforming protein 53 (p53)</i>	17p13.1	Lohmann <i>et al.</i> (1993a,b)
<i>RB</i>	13q14.1–q14.2	Harbour <i>et al.</i> (1988), Hiyama <i>et al.</i> (1993)

^aSDHD is unlikely to be involved as the reported alterations are polymorphisms.

gains and losses in the tumour genome can be detected by the use of comparative genomic hybridisation (CGH), which is a competitive *in situ* hybridisation-based procedure that reveals relative gene copy aberrations (Fig. 1) (Kallioniemi *et al.* 1992, du Manoir *et al.* 1993). Changes in relative gene copy number detected using CGH, which may be associated with oncogene amplification or loss of tumour-suppressor gene function, deleted in one non-familial bronchial NET are shown in Fig. 2.

The *MEN1* gene and NETs

NETs are commonly seen in patients with MEN1, which is an autosomal dominant disorder that is characterised by the combined occurrence of tumours of the parathyroid, pancreatic

islet cells (e.g. gastrinomas, insulinomas, vasoactive intestinal polypeptidomas (VIPomas), glucagonomas, pancreatic polypeptidomas (PPomas) and non-functioning tumours), and anterior pituitary (e.g. prolactinomas, somatotrophinomas, corticotrophinomas and non-functioning tumours) (Thakker 2000, Pannett & Thakker 2001). Other associated tumours may also arise in MEN1 patients and these include adrenal cortical tumours, bronchial NETs, thymic NETs, lipomas, collagenomas and angiofibromas (Trump *et al.* 1996). The *MEN1* gene on chromosome 11q13 represents a putative tumour suppressor gene, consistent with the Knudson model for tumour development (Knudson 1974, Pannett & Thakker 2001). The *MEN1* gene consists of 10 exons which span >9 kb of genomic DNA (Chandrasekharappa *et al.* 1997, The European Consortium on MEN1 1997) (Fig. 3). The 1.83

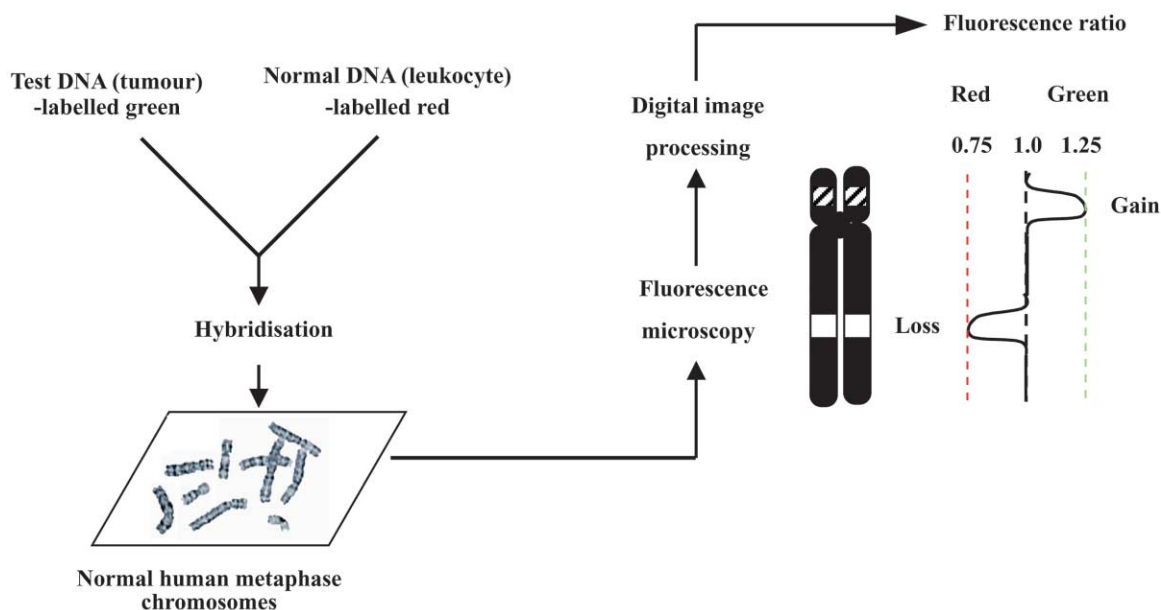


Figure 1 Differentially labelled test DNA and normal reference DNA are hybridised simultaneously to normal chromosome spreads. The hybridisation is detected with two different fluorochromes (green and red). Regions of gain (hatched boxes) due to duplications or amplifications of DNA sequences, or regions of loss (open boxes) due to deletions, are seen as changes in the ratio of the intensities of the two fluorochromes along the target chromosomes (Kallioniemi *et al.* 1993, du Manoir *et al.* 1993).

kb mRNA encodes a novel 610 amino acid protein referred to as MENIN (Chandrasekharappa *et al.* 1997). Over 600 germline mutations of the *MEN1* gene have been identified and these are scattered throughout the coding region and are of diverse types (Pannett & Thakker 1999, Turner *et al.* 2002). Thus, approximately 21% are nonsense, 44% are frameshift deletions or insertions, 9% are in-frame deletions or insertions, 7% are splice site mutations and 19% are missense mutations (Turner *et al.* 2002). Such germline *MEN1* mutations are frequently associated with NETs and this is illustrated by family 7/03 (Fig. 4), in which affected members have a missense *MEN1* mutation and develop different NETs that include gastrinomas, insulinomas and lung NETs (Trump *et al.* 1996, The European Consortium on MEN1 1997). The majority of *MEN1* tumours have LOH of chromosome 11q13 and a few have been shown to harbour somatic mutations consistent with the Knudson two-hit hypothesis (Pannett & Thakker 2001). In addition, LOH at the *MEN1* locus on chromosome 11q13 and somatic *MEN1* mutations have also been observed in sporadic NETs of foregut origin, thereby indicating a role for the *MEN1* gene in the aetiology of such tumours (Toliat *et al.* 1997, Zhuang *et al.* 1997b, Hessman *et al.* 1998, Shan *et al.* 1998, Wang *et al.* 1998, Fujii *et al.* 1999, Gortz *et al.* 1999, Mailman *et al.* 1999, Bergman *et al.* 2000, Goebel *et al.* 2000, Pannett & Thakker 2001). Thus, LOH at chromosome 11q13 has been shown in approximately 60% of sporadic foregut NETs and pancreatic islet-cell tumours, which include gastrinomas,

insulinomas, VIPomas and glucagonomas (Jakobovitz *et al.* 1996, Debelenko *et al.* 1997a, Dong *et al.* 1997, Emmert-Buck *et al.* 1997, Toliat *et al.* 1997, D'Adda *et al.* 1999b). In addition, somatic *MEN1* mutations have, to date, been reported in 11 sporadic foregut NETs, excluding pancreatic NETs (Table 4 and Fig. 3) (Debelenko *et al.* 1997a, 2000, Baudin *et al.* 1999, Fujii *et al.* 1999, Gortz *et al.* 1999). The mutations consist of three nonsense mutations, six frameshift deletions and insertions, one missense mutation and one donor splice site mutation. The nonsense, frameshift and donor splice site mutations are predicted to lead to truncated MENIN proteins, and these and the form resulting from the missense mutation are likely to disrupt the functions of MENIN (see below) by altering its binding to interacting proteins (Fig. 5).

Function of MENIN

MENIN is predominantly a nuclear protein in non-dividing cells, but in dividing cells it is also found in the cytoplasm (Guru *et al.* 1998). The function of MENIN still remains to be elucidated, but it has been shown to interact with a number of proteins that are involved in transcriptional regulation and the control of genome stability. Thus, in transcriptional regulation, MENIN has been shown to interact with: (i) the activating protein-1 transcription factors JunD (Agarwal *et al.* 1999) and c-Jun (Yumita *et al.* 2003) to suppress Jun-mediated transcriptional activation; (ii) members of

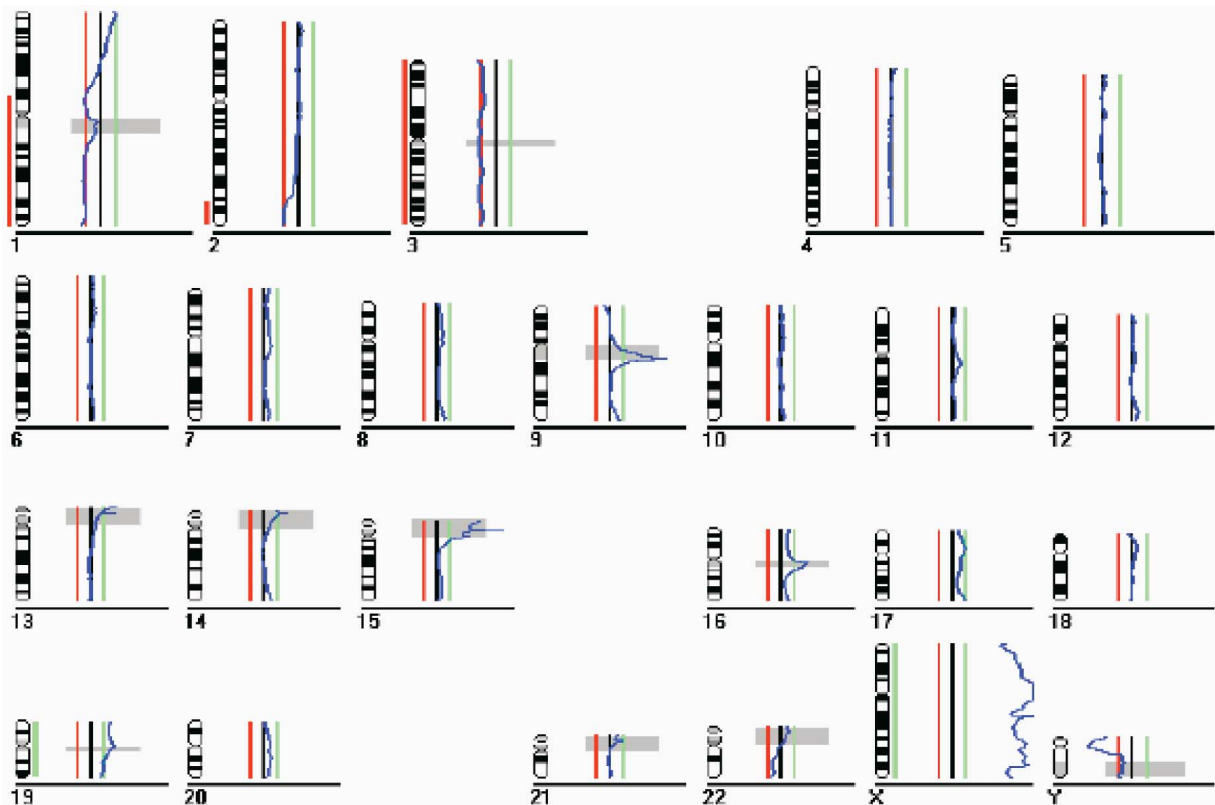


Figure 2 CGH analysis of a lung NET from a female patient 5 (see Table 5). The fluorescence ratio profiles, which indicate the chromosomal differences, are shown to the right of the chromosomes. The left-hand line represents a fluorescence ratio of 0.75 (i.e. loss), the centre line a ratio of 1.0 (i.e. normal) and the right-hand line a ratio of 1.25 (i.e. gain) (Fig. 1). The tumour DNA is from a female (46XX), and has been hybridised to metaphase chromosomes from a male (46XY), and hence an 'apparent' gain of the X chromosome (fluorescence ratio > 1.25) and an 'apparent' loss of the Y chromosome (fluorescence ratio < 0.75) are observed. In addition, 'apparent' gains are sometimes observed around the centromeric regions, which contain repetitive elements, e.g. chromosomes 9 and 6. Thus, the tumour reveals a 'true' loss of chromosomes 1q21-qter, 2q34-qter and 3, and a 'true' gain of chromosome 19.

the NF- κ B family (e.g. p50, p52 and p65) of transcription regulators to repress NF- κ B-mediated transcriptional activation (Heppner *et al.* 2001); (iii) members of the Smad family (Smad 3, Smad1/5 complex) to modulate the transforming growth factor- β (TGF β) pathway (Kaji *et al.* 2001) and the bone morphogenetic protein-2-signalling pathways, respectively (Sowa *et al.* 2003); and (iv) the mouse placenta and embryonic expression (*Pem*) gene, which encodes a homeobox containing protein that plays a role in transcription (Lemmens *et al.* 2001). A role for MENIN in controlling genome stability has been reported because it interacts with: (i) a subunit of RPA2, which is a heterotrimeric protein required for DNA replication, recombination and repair (Sukhodolets *et al.* 2003); (ii) the tumour metastases suppressor NM23H1/nucleoside diphosphate kinase (Ohkura *et al.* 2001, Yaguchi *et al.* 2002), which induces GTPase activity; and (iii) the glial fibrillary acidic protein and vimentin (Lopez-Egido *et al.* 2002), which are involved in the

intermediate filament network. The 11 somatic MEN1 mutations reported in the sporadic NETs (Table 4) are predicted to disrupt these protein interactions (Fig. 5) and hence the function of MENIN in regulating cell proliferation.

Other genes involved in NETs

Foregut NETs

The NETs that have been studied for genetic abnormalities are principally from the lung (bronchial), thymus, stomach and pancreas and each has the involvement of different genes.

Lung NETs

Lung NETs represent a broad spectrum of morphological types, ranging, in order of aggressiveness and malignant potential, from typical carcinoids (TC), atypical carcinoids

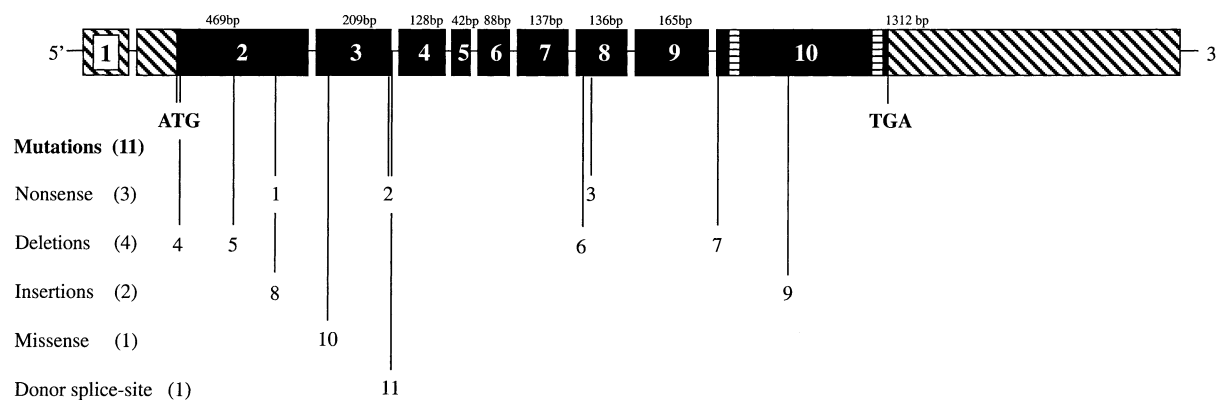


Figure 3 Schematic representation of the genomic organisation of the *MEN1* gene illustrating 11 somatic mutations found in non-familial, i.e. sporadic isolated (i.e. non-*MEN1* patients) bronchial, thymic, gastric and duodenal NETs (see Table 4). The human *MEN1* gene consists of 10 exons that span > 9 kb of genomic DNA and encodes a 610 amino acid protein (Chandrasekharappa *et al.* 1997, The European Consortium on *MEN1* 1997). The 1.83 kb coding region is organised into 9 exons (exons 2–10) and 8 introns (indicated by a line but not to scale). The sizes of the exons (solid boxes) range from 42 to 1312 bp and those of the introns range from 41 to 1564 bp. The start (ATG) and stop (TGA) sites in exons 2 and 10 respectively, are indicated. Exon 1, the 5' part of exon 2 and 3' part of exon 10 are untranslated (indicated by hatched boxes). The locations of the two nuclear localisation sites, which are at codons 479 to 497, and 588 to 608 at the C-terminus, are represented by horizontally hatched boxes in exon 10. The sites of the 11 somatic mutations (three nonsense mutations, six frameshift mutations, one missense mutation and one donor splice site mutation) found in sporadic NETs are represented below the gene, and details of each of these are provided in Table 4.

(AC), higher grade LCNECs, to SCLCs. LOH involving chromosomes 3p, 5q21, 9p, 11q13 (*MEN1* gene, see above), 13q13 (*RB* gene) and 17p13 have been observed in lung NETs. These results of LOH studies are broadly confirmed by our CGH analysis of seven lung NETs that were all bronchial TCs, in which chromosome 3 loss was observed in two of the tumours and chromosome 9 loss was observed in one of the tumours (Table 5). Interestingly, our CGH studies reveal a gain of chromosome 19 in five of the lung NETs and these results suggest that amplification of genes on chromosome 19 may be important in the aetiology of foregut NETs. However, another study of 11 lung NETs found loss of chromosome 11q in 36% of tumours (Zhao *et al.* 2000). The other abnormalities that were found in approximately 10% of lung NETs included gains of chromosomes 5, 7, 8, 9q, 14q, 15q, 16q, 17 and 20q, and losses of chromosome 1p, 6q and 22q (Zhao *et al.* 2000). Further studies of such tumours are required to clarify the chromosomal abnormalities associated with these foregut NETs.

LOH at chromosome 3p is the most frequent change in lung NETs and has been observed in 40% of TC, 73% of AC, 83% of LCNEC and 85% of SCLC (Onuki *et al.* 1999). The *FHIT* locus at chromosome 3p14.2 has been particularly studied and LOH was observed in 23% of TC, 35%-50% of AC, 80% of LCNEC and 67% of SCLC, but no somatic mutations have been reported (Kovatch *et al.* 1998, Onuki *et al.* 1999). The frequency of LOH on chromosome 5q21, which is the location of the *APC/MCC* genes, increases with

the severity of the tumour. Thus, LOH at chromosome 5q21 was observed in 0% of TC, 25% of AC, 46% of LCNEC and 86% of SCLC and was correlated with a poor survival in patients with lung NETs (Onuki *et al.* 1999). Furthermore, a meta-analysis using data from the literature revealed that LOH at chromosome 5q in lung neuroendocrine carcinomas (LCNEC and SCLC) was higher, at 75%, than in lung carcinoid tumours (TC and AC) in which the frequency was 1.4% (Ullmann *et al.* 2002). LOH at chromosome 9p21, which is the location of the *p16* locus, is also higher in carcinomas (53%) than in the carcinoid tumours (23%) (Onuki *et al.* 1999). LOH and point mutations of the *p53* locus on chromosome 17p13 were detected in 10% of TC (Lohmann *et al.* 1993a, Ramnani *et al.* 1999), 45% of AC (Hiyama *et al.* 1993, Couce *et al.* 1999), 72% of LCNEC (Lohmann *et al.* 1993a, Couce *et al.* 1999) and 90% of SCLC (Sameshima *et al.* 1992). Furthermore, these *p53* alterations in lung NETs were found to correlate with poor survival rates amongst patients (Onuki *et al.* 1999). LOH at the *RB* locus on chromosome 13q14.1-q14.2 also increased with the aggressiveness of the tumour such that there was a demarcation between the lung carcinoids (AC and TC) and the carcinomas (LCNEC and SCLC), thereby suggesting that different mechanisms may be involved in tumorigenesis in these two types of lung NETs (Onuki *et al.* 1999). Furthermore, two mutations of *K-ras*, which is a dominant oncogene that is aberrant in most human tumours including lung cancer (Nakano *et al.* 1984), have been found in atypical lung NETs (Couce *et al.* 1999). Overall,

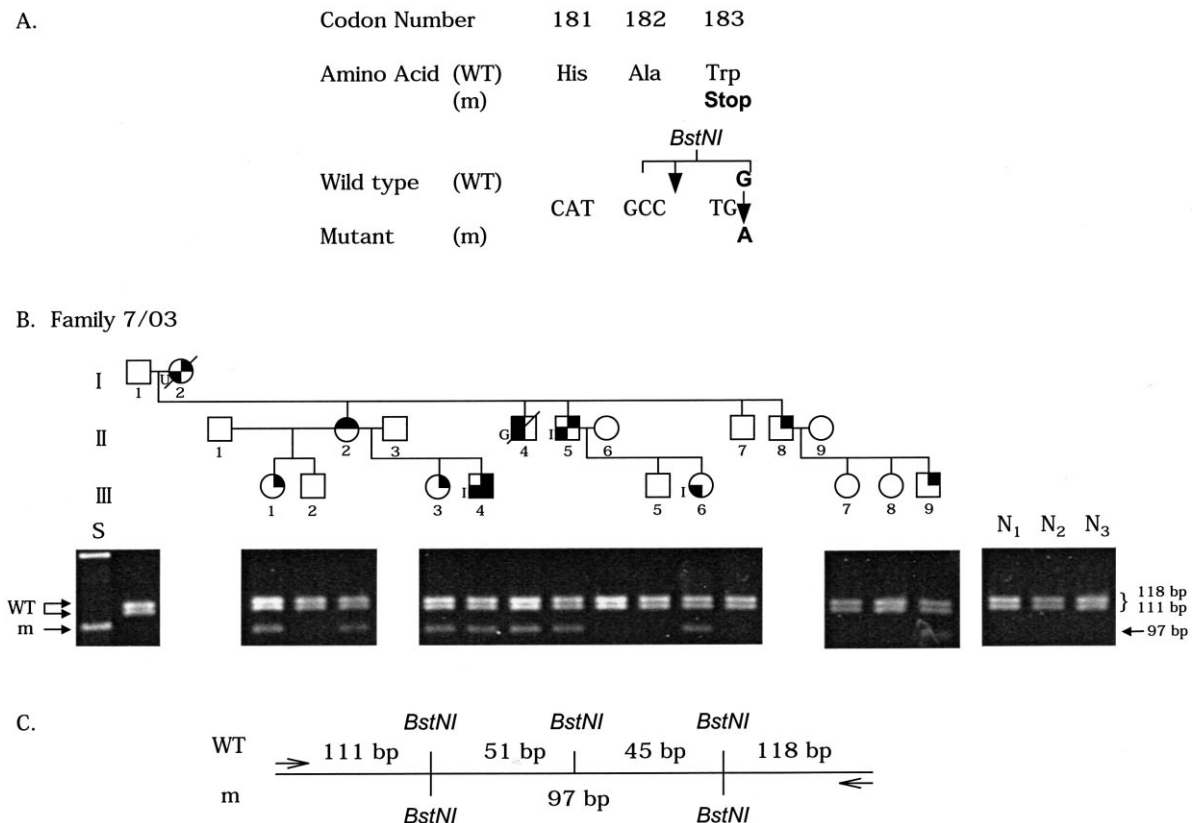


Figure 4 Detection of *MEN1* mutation (Trp183Stop) in exon 3 in family 7/03 by restriction enzyme analysis. DNA sequence analysis of individual II-5 revealed a G → A transition at the third nucleotide of codon 183. The transition results in the loss of a *BstNI* restriction enzyme site (CC/TGG) from the WT sequence (A), and this has facilitated the detection of the mutation in the other affected members (II.2, II.4, II.5, III.1, III.3, III.4, III.6 and III.9) of this family (B). The mutant (m) PCR product is 97 bp (C), whereas the WT products are 111, 118, 51 and 45 bp. The 51 and 45 bp products are not shown. The affected individuals are heterozygous (WT/m), and the unaffected family members (I.1, II.6, II.7, III.2, III.5, III.7 and III.8) and control unrelated normals (N1–N3) are homozygous for the WT allele (B). Individuals are represented as males (squares), females (circles), unaffected (open symbols), affected with parathyroid tumours (solid upper right quadrant), affected with corticotrophinoma (solid lower right quadrant), affected with pancreatic tumour (solid lower left quadrant; G, gastrinoma, I, insulinoma and U, unknown) and affected with lung NET (solid upper left quadrant). The positions of the size markers (S) at 100 bp and 200 bp are shown. Cosegregation of this mutation with MEN1 in family 7/03 and its absence from 55 unrelated normal individuals (N1–N3 shown) was demonstrated, thereby indicating that it is not a common polymorphism. The clinical findings in this family, 7/03, with the Trp183Stop mutation have been updated from those previously reported as family 7/87 (Trump *et al.* 1996, The European Consortium on MEN1 1997).

these studies of lung NETs reveal four important findings: (i) LOH at chromosome 3p is the most frequent change in these tumours; (ii) LOH of the *MEN1* locus is also common; (iii) LOH and mutations of p53 cumulatively increase with severity of the tumour type; and (iv) LOH at 5q21, the location of the *APC* and *MCC* genes is correlated with a poor survival.

In addition to these studies of LOH, CGH and mutational analysis, lung NETs have also been investigated for silencing of genes by methylation. Genes that are frequently methylated in gastrointestinal malignancies were investigated in lung TCs, ACs, SCLCs and NSCLC, and the frequencies of

methylation of the *RARβ*, *CDH1*, *RASSF1A* and *CASP8* genes were found to be higher in SCLC (45%, 55%, 80% and 35% respectively) than in TCs and ACs (20%, 25%, 55% and 18% respectively), although ACs seemed to have a higher frequency (71%) of *RASSF1A* methylation when compared with TCs (45%). However, methylation of the *RASSF1A*, *p16*, *APC* and *CDH13* genes was significantly higher in NSCLCs when compared with the lung NETs (TC, AC and SCLC) (Toyooka *et al.* 2001, Shivapurkar *et al.* 2002). These methylation differences between lung TCs, ACs, SCLCs and NSCLC reinforce the view that these tumours are fundamentally different and that the tumorigenic

Table 4 Somatic *MEN1* mutations in sporadic foregut NETs, excluding those of the pancreas^a

Mutation type; number	Exon	Codon	Base change	Predicted effect ^b	Tissue	Reference
Nonsense						
1	Exon 2	108	CGA → TGA	ns, Arg108 Stop	Bronchial	Baudin <i>et al.</i> (1999)
2	Exon 3	209	CAG → TAG	ns, Gln209 Stop	Duodenal	Gortz <i>et al.</i> (1999)
3	Exon 8	393	CAA → TAA	ns, Gln393 Stop	Thymic	Fujii <i>et al.</i> (1999)
Deletion						
4	Exon 2	8–12	nt134del13bp	fs, ins 108 aa Stop	Bronchial	Debelenko <i>et al.</i> (1997a)
5	Exon 2	83–84	nt357del4p	fs, ins 34 aa Stop	Stomach ^c	Fujii <i>et al.</i> (1999)
6	Exon 8	372	nt1226delC	fs, ins 3 aa Stop	LCNEC ^d	Debelenko <i>et al.</i> (2000)
7	Exon 10	451	nt1461delG	fs, ins 7 aa Stop	Bronchial	Debelenko <i>et al.</i> (1997a)
Insertion						
8	Exon 2	108	nt134ins29bp	fs, ins 10 aa Stop	Bronchial	Gortz <i>et al.</i> (1999)
9	Exon 10	514	nt1650insC	fs, ins 44 aa Stop	Bronchial	Debelenko <i>et al.</i> (1997a)
Missense						
10	Exon 3	172	GAT → TAT	ms, Asp172Val	Bronchial	Gortz <i>et al.</i> (1999)
Donor splice site mutation						
11	Intron 3		nt764+3 (ag)	ds, fs, ins 18 aa Stop	Bronchial	Debelenko <i>et al.</i> (1997a)

^aSomatic *MEN1* mutations have been found in sporadic pancreatic NETs, including gastrinomas (Zhuang *et al.* 1997a, Wang *et al.* 1998, Mailman *et al.* 1999, Goebel *et al.* 2000), insulinomas (Zhuang *et al.* 1997a, Shan *et al.* 1998, Pannett & Thakker 2001), VIPomas (Shan *et al.* 1998, Wang *et al.* 1998, Gortz *et al.* 1999) and glucagonomas (Hessman *et al.* 1998, Bergman *et al.* 2000). Midgut and hindgut NETs generally do not have *MEN1* mutations, though a missense mutation, Val53Ile, has been identified in one ileal NET (Gortz *et al.* 1999).

^bPredicted effect: fs, frameshift mutation; ns, nonsense mutation; ms, missense mutation; ds, donor splice site mutation; ins, insertion; aa, amino acid.

^cGastric neuroendocrine carcinoma (Fujii *et al.* 1999).

^dLCNEC, large cell neuroendocrine carcinoma. Mutations are numbered with reference to the *MEN1* cDNA sequence U93236 (GenBank).

process in each type evolves along different pathways with tumours of the same histological type having similar methylation patterns. Furthermore, methylation of the *death-associated protein kinase* and *APC* genes in lung cancers has also been shown to correlate with poor survival (Tang *et al.* 2000, Virmani *et al.* 2001). These studies are important as they may have a potential impact on the clinical management of patients, as treatment with demethylating agents, which results in a temporary restoration of gene expression, may represent a potential method of tumour therapy or chemoprevention (Mompalmer *et al.* 2000, Schwartzmann *et al.* 2000) in patients who have tumours with such methylated genes.

Thymic NETs

The genetic abnormalities in thymic NETs have not been as well characterised as the other foregut NETs. Twenty five per cent of reported thymic NETs are from *MEN1* patients (Teh 1998), and LOH of the *MEN1* locus on chromosome 11q13 has not been reported in thymic NETs, though a somatic *MEN1* mutation has been reported in one thymic

NET (Teh 1998, Fujii *et al.* 1999, Hessman *et al.* 2001). LOH of chromosome 1p has been reported in two thymic NETs (Teh *et al.* 1998), and our CGH results (Table 5) reveal the occurrence of deletions of chromosomes 3, 9p21-pter, and Y and a gain of chromosome 19p in a thymic NET from a male patient.

Gastric NETs

Gastric NETs are derived from the enterochromaffin-like (ECL) cell. Four main types referred to as ECL types I–III (ECL-I to -III) and neuroendocrine carcinoma are recognised depending upon their clinical and pathological characteristics (Rindi *et al.* 1993, Bordi 1995). ECL-I tumours, which are associated with atrophic gastritis and hypergastrinaemia, account for 70–80% of all ECLomas and consist of small, multiple tumours that are limited to the mucosa or submucosa, and rarely metastasise to regional lymph nodes. More than 85% of ECL-I tumours are monoclonal (D'Adda *et al.* 1999a). ECL-II tumours, which are associated with *MEN1* and the Zollinger–Ellison syndrome, account for 6% of ECLomas and consist of multiple tumours that infiltrate to

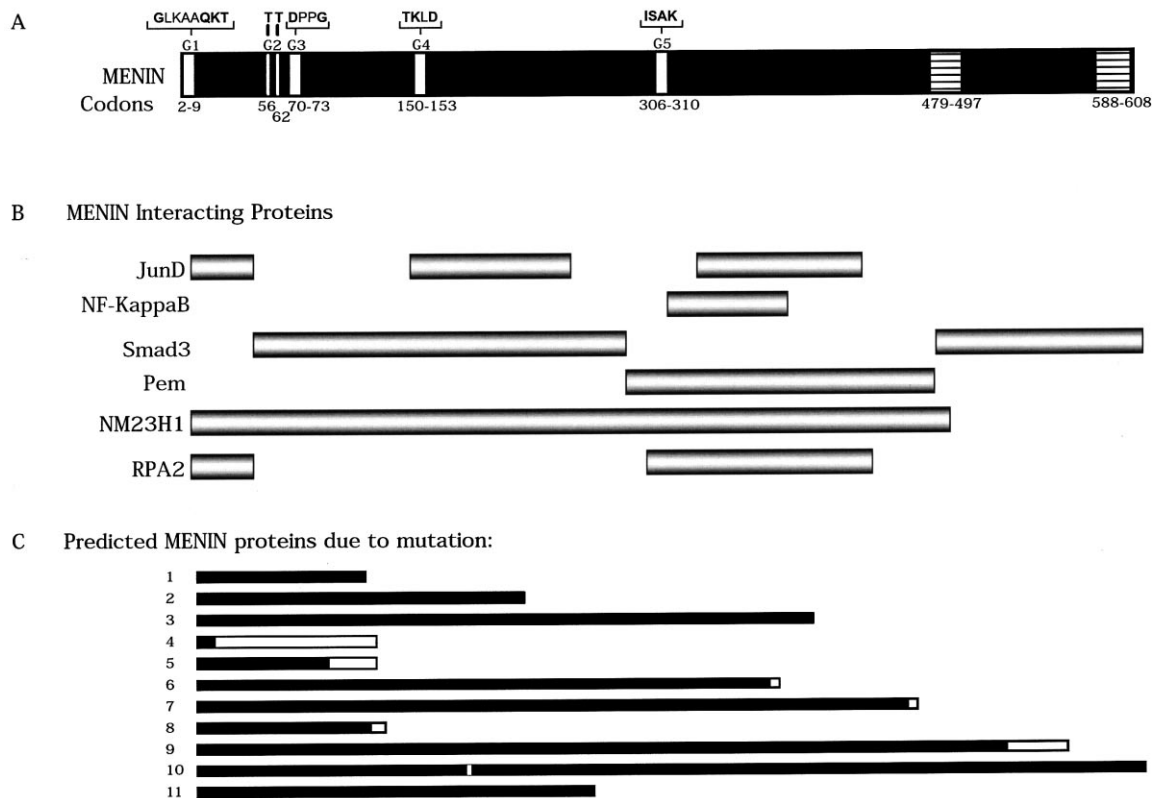


Figure 5 Schematic representation of the amino acid sequence of MENIN together with the regions that interact with other proteins. (A) MENIN has two nuclear localisation signals (horizontally hatched boxes) at codons 479–497 and 588–608 (Guru *et al.* 1998), and 5 putative GTPase sites (G1–G5) (open boxes), whose consensus sequences are shown above, with the conserved amino acid residues shown in bold (Yaguchi *et al.* 2002). (B) MENIN regions that likely interact with JunD (codons: 1–40, 139–242, 323–428) (Agarwal *et al.* 1999); nuclear factor- κ B (NF- κ B)) (codons: 305–381) (Heppner *et al.* 2001); small-mothers against decapentaplegic homology 3, Smad3 (codons: 40–278, 477–610) (Kaji *et al.* 2001); placenta and embryonic expression, Pem (codons: 278–476) (Lemmens *et al.* 2001); non-metastatic 23 β human genes, NM23H1 (codons: 1–486) (Ohkura *et al.* 2001); and a subunit of replication protein A (RPA2) (codons 1–40, 286–448) (Sukhodolets *et al.* 2003) are indicated by the boxes with grey shading. (C) Truncated or other abnormal forms of MENIN that are predicted to result from the 11 somatic *MEN1* mutations reported in sporadic foregut NETs, excluding pancreatic neuroendocrine tumours, are represented by bars (normal sequence, solid bars and missense sequences, open bars).

Table 5 CGH analysis of eight NETs (seven lung TCs and a thymic NET)

Patient no.	Sex ^a	NET	Chromosomal losses ^b	Chromosomal gains
1	M	Lung TC ^d	11q12-pter, 11p13-pter	19
2	M	Lung TC	22q13-qter	13, 19
3	F	Lung TC		14, 17, 19, 20q, 22
4	F	Lung TC		5, 19
5	F	Lung TC	1p21-qter, 2q34-qter, 3	19
6	F	Lung TC	1q13-p31, 1q32-q41, 5, 4, 6q, 9p, 17p, X, 3q13-p14, 8q24-pter, 13q21-qter, 15q13-q21, 18q22-qter, 20p12-pter	8p23-qter, 17q
7 ^c	M	Lung TC		19p, X
8 ^c	M	Thymus	3, 9p21-pter, Y	19p

^aSex: M, Male; F, Female.

^bp, Short arm of a chromosome; q, long arm of a chromosome; ter, telomeric.

^cThe lung nodule and the thymic NET were from the same patient.

^dTC, typical carcinoid.

the submucosa. The prognosis for ECL-II tumours is generally good, even if they occasionally become aggressive (Rindi *et al.* 1993, Bordi 1995). ECL-III tumours, which are associated with hypergastrinaemia, account for 14–25% of ECLomas and usually consist of a single tumour that is often infiltrating the muscularis propria and serosa. ECL-III tumours, which may have LOH of the X chromosome (D'Adda *et al.* 1999a), frequently metastasise, and hepatic metastases occur in 50% of patients who have a poor prognosis. Neuroendocrine carcinomas, which are uncommon, are usually single, large, poorly differentiated and highly malignant, and LOH on chromosomes 8p, 11p, 12p, 13q, 15q and 17p has been reported in 82%, 50%, 50%, 50%, 58% and 57% of 15 such tumours respectively (Han *et al.* 2000).

LOH of the *MEN1* locus on chromosome 11q13 has been found in 48% of 25 ECL-I tumours (D'Adda *et al.* 1999b), 75% of 20 ECL-II tumours (Debelenko *et al.* 1997b) and both of two neuroendocrine carcinomas (D'Adda *et al.* 1999b). Somatic *MEN1* mutations have not been detected in type I and III ECLomas, but a 4 bp deletion at codon 83 of the *MEN1* gene that results in a frameshift and truncated protein (Table 4 and Figs 3 and 5) has been reported in one gastric neuroendocrine carcinoma (Fujii *et al.* 1999).

Pancreatic NETs

All pancreatic NETs from *MEN1* patients have shown a loss (LOH) of chromosome 11. However, 30% of these tumours may also show LOH at chromosomes 3, 6, 8, 10, 18 and 21 (Hessman *et al.* 2001). In addition, LOH at chromosomes 3 and 6 is also observed in >50% and >60% of sporadic (i.e. non *MEN1*) pancreatic NETs respectively. The chromosome 3 losses particularly occur as late events, thereby suggesting that putative tumour suppressor genes on this chromosome may be involved in a more aggressive tumour phenotype (Barghorn *et al.* 2001, Guo *et al.* 2002). Furthermore, a point mutation of the *N-Ras* gene has been identified in a PET (Arany *et al.* 1994).

Midgut NETs (carcinoids)

In contrast to foregut NETs, which have frequent deletions and mutations of the *MEN1* gene, midgut NETs (i.e. carcinoids) rarely show involvement of the *MEN1* gene. However, CGH studies have shown losses of chromosome 11q in 22% of ileal and duodenal carcinoids, and this has involved the distal part of chromosome 11q, where the tumour suppressor gene *SDHD* is located (Kytola *et al.* 2002). Germline mutations of *SDHD* have been reported in families with hereditary head and neck paragangliomas linked to 11q23, and in families with adrenal and extra-adrenal pheochromocytomas (Baysal *et al.* 2000, Gimm *et al.* 2000, Astuti *et al.* 2001a,b, Badenhop *et al.* 2001, Milunsky *et al.* 2001, Taschner *et al.* 2001). Two possible germline missense mutations (His50Arg and Gly12Ser) of the *SDHD*

gene were reported in two sporadic midgut carcinoid tumours, which also had LOH at the *SDHD* locus on chromosome 11q23 (Kytola *et al.* 2002). There is debate about whether these changes are mutations or polymorphisms, particularly as Gly12Ser has been reported in a phenotypically normal patient (Gimm *et al.* 2000) and it has also been reported that His50Arg may represent a rare polymorphism. Furthermore, *SDHD* mutations have not been detected in 45 NETs of the lung, gastrointestinal, pancreatic and parathyroid (Perren *et al.* 2001), and thus it would seem unlikely that the *SDHD* gene is involved in midgut NETs which are likely to be due to other genes. Indeed CGH studies of midgut carcinoids have shown losses of chromosomes 9p, 18p and 18q in 21%, 38% and 33% respectively (Zhao *et al.* 2000, Tonnie *et al.* 2001), gains of chromosomes 17q and 19p in 57% (Tonnie *et al.* 2001) and LOH of chromosome 18 has been reported in 88% of midgut NETs (Lollgen *et al.* 2001). In addition, 22% of midgut tumours have losses of 16q21 and metastases show an accumulation of genetic abnormalities, which particularly involve a loss of chromosome 16q and a gain of chromosome 4p. Furthermore, a *K-ras* mutation, Gly12Asp, has been identified in an extra-hepatic bile duct NET (Maitra *et al.* 2000). These findings indicate that midgut and foregut NETs are likely to develop via different molecular pathways, although the genes involved at chromosomes 4p, 9p, 16q, 18p and 18q, in causing midgut carcinoids, remain to be identified. Appendiceal goblet-cell carcinoids may have yet another molecular pathway of development as up-regulation of cyclin D1 and p21 together with down-regulation of p16 have been reported (Kanthan *et al.* 2001, Oberg 2002).

Hindgut NETs

The genetic abnormalities in hindgut NETs have not been as well characterised as those in foregut and midgut NETs, and LOH of chromosome 18 has been identified in only one ascending colon NET (Lollgen *et al.* 2001). Rectal NETs that are >5 mm in size have been reported to express TGF α more frequently, and the epidermal growth factor (EGF) receptor was expressed in all lesions (Shimizu *et al.* 2000). It has therefore been proposed that TGF α and the EGF receptor may participate in an autocrine mechanism that regulates the growth of hindgut NETs, and hence these hindgut tumours are likely to develop via different molecular pathways from those of the foregut and midgut NETs (Shimizu *et al.* 2000).

Conclusion

NETs may occur as part of complex familial endocrine cancer syndromes such as *MEN1* and *NF1*, although the majority occur as non-familial (i.e. sporadic) isolated tumours. Different genes are involved in the aetiology of each type of NET and different genetic abnormalities that

include point mutations, gene deletions, DNA methylation, chromosomal losses and chromosomal gains may be involved. Furthermore, the foregut, midgut and hindgut NETs develop via different molecular pathways. For example, foregut NETs have frequent deletions and mutations of the *MEN1* gene, whereas midgut NETs have losses of chromosomes 18, 11q and 16q, and hindgut NETs, express TGF α and the EGF receptor. In addition, in lung NETs, a loss of chromosome 3p is the most frequent change, and p53 mutations and chromosomal loss of 5q21 are associated with more aggressive tumours and poor survival. Moreover, methylation frequencies of *RAR β* , *CDHI* and *RASSF1A* genes increase with the severity of lung NETs. Thus the development and progression of NETs is associated with specific genetic abnormalities that indicate the likely involvement of different molecular pathways.

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References

- Agarwal SK, Guru SC, Heppner C, Erdos MR, Collins RM, Park SY, Saggar S, Chandrasekharappa SC, Collins FS, Spiegel AM, Marx SJ & Burns AL 1999 Menin interacts with the API transcription factor JunD and represses JunD-activated transcription. *Cell* **96** 143–152.
- Anderson RE 1966 A familial instance of appendiceal carcinoid. *American Journal of Surgery* **111** 738–740.
- Arany I, Rady P, Evers BM, Tyring SK & Townsend CM Jr 1994 Analysis of multiple molecular changes in human endocrine tumours. *Surgical Oncology* **3** 153–159.
- Astuti D, Douglas F, Lennard TW, Aligianis IA, Woodward ER, Evans DG, Eng C, Latif F & Maher ER 2001a Germline SDHD mutation in familial pheochromocytoma. *Lancet* **357** 1181–1182.
- Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C & Maher ER 2001b Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *American Journal of Human Genetics* **69** 49–54.
- Badenhop RF, Cherian S, Lord RS, Baysal BE, Taschner PE & Schofield PR 2001 Novel mutations in the SDHD gene in pedigrees with familial carotid body paraganglioma and sensorineural hearing loss. *Genes Chromosomes and Cancer* **31** 255–263.
- Barghorn A, Komminoth P, Bachmann D, Rutimann K, Saremaslani P, Muletta-Feurer S, Perren A, Roth J, Heitz PU & Speel EJ 2001 Deletion at 3p25.3-p23 is frequently encountered in endocrine pancreatic tumours and is associated with metastatic progression. *Journal of Pathology* **194** 451–458.
- Baudin E, Bidart JM, Rougier P, Lazar V, Ruffie P, Ropers J, Ducreux M, Troalen F, Sabourin JC, Comoy E, Lasser P, DeBaere T & Schlumberger M 1999 Screening for multiple endocrine neoplasia type 1 and hormonal production in apparently sporadic neuroendocrine tumors. *Journal of Clinical Endocrinology and Metabolism* **84** 69–75.
- Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW III, Cornelisse CJ, Devilee P & Devlin B 2000 Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287** 848–851.
- Bergman L, Boothroyd C, Palmer J, Grimmond S, Walters M, Teh B, Shepherd J, Hartley L & Hayward N 2000 Identification of somatic mutations of the MEN1 gene in sporadic endocrine tumours. *British Journal of Cancer* **83** 1003–1008.
- Bird AP 1986 CpG-rich islands and the function of DNA methylation. *Nature* **321** 209–213.
- Bordi C 1995 Endocrine tumours of the stomach. *Pathology, Research and Practice* **191** 373–380.
- Brambilla E, Travis WD, Colby TV, Corrin B & Shimosato Y 2001 The new World Health Organisation classification of lung tumours. *European Respiratory Journal* **18** 1059–1068.
- Brown MA & Solomon E 1997 Studies on inherited cancers: outcomes and challenges of 25 years. *Trends in Genetics* **13** 202–206.
- Chan AO, Kim SG, Bedeir A, Issa JP, Hamilton SR & Rashid A 2003 CpG island methylation in carcinoid and pancreatic endocrine tumors. *Oncogene* **22** 924–934.
- Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL & Marx SJ 1997 Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* **276** 404–407.
- Couce ME, Bautista D, Costa J & Carter D 1999 Analysis of K-ras, N-ras, H-ras, and p53 in lung neuroendocrine neoplasms. *Diagnostic Molecular Pathology* **8** 71–79.
- D'Adda T, Candidus S, Denk H, Bordi C & Hofler H 1999a Gastric neuroendocrine neoplasms: tumour clonality and malignancy-associated large X-chromosomal deletions. *Journal of Pathology* **189** 394–401.
- D'Adda T, Keller G, Bordi C & Hofler H 1999b Loss of heterozygosity in 11q13–14 regions in gastric neuroendocrine tumors not associated with multiple endocrine neoplasia type 1 syndrome. *Laboratory Investigation* **79** 671–677.
- Dammann R, Takahashi T & Pfeifer GP 2001 The CpG island of the novel tumor suppressor gene RASSF1A is intensely methylated in primary small cell lung carcinomas. *Oncogene* **20** 3563–3567.
- Debelenko LV, Brambilla E, Agarwal SK, Swalwell JI, Kester MB, Lubensky IA, Zhuang Z, Guru SC, Manickam P, Olufemi SE, Chandrasekharappa SC, Crabtree JS, Kim YS, Heppner C, Burns AL, Spiegel AM, Marx SJ, Liotta LA, Collins FS, Travis WD & Emmert-Buck MR 1997a Identification of MEN1 gene mutations in sporadic carcinoid tumors of the lung. *Human Molecular Genetics* **6** 2285–2290.
- Debelenko LV, Emmert-Buck MR, Zhuang Z, Epshteyn E, Moskaluk CA, Jensen RT, Liotta LA & Lubensky IA 1997b The multiple endocrine neoplasia type I gene locus is involved in the

- pathogenesis of type II gastric carcinoids. *Gastroenterology* **113** 773–781.
- Debelenko LV, Swalwell JI, Kelley MJ, Brambilla E, Manickam P, Baibakov G, Agarwal SK, Spiegel AM, Marx SJ, Chandrasekharappa SC, Collins FS, Travis WD & Emmert-Buck MR 2000 MEN1 gene mutation analysis of high-grade neuroendocrine lung carcinoma. *Genes Chromosomes and Cancer* **28** 58–65.
- Dong Q, Debelenko LV, Chandrasekharappa SC, Emmert-Buck MR, Zhuang Z, Guru SC, Manickam P, Skarulis M, Lubensky IA, Liotta LA, Collins FS, Marx SJ & Spiegel AM 1997 Loss of heterozygosity at 11q13: analysis of pituitary tumors, lung carcinoids, lipomas, and other uncommon tumors in subjects with familial multiple endocrine neoplasia type 1. *Journal of Clinical Endocrinology and Metabolism* **82** 1416–1420.
- Emmert-Buck MR, Lubensky IA, Dong Q, Manickam P, Guru SC, Kester MB, Olufemi SE, Agarwal S, Burns AL, Spiegel AM, Collins FS, Marx SJ, Zhuang Z, Liotta LA, Chandrasekharappa SC & Debelenko LV 1997 Localization of the multiple endocrine neoplasia type I (MEN1) gene based on tumor loss of heterozygosity analysis. *Cancer Research* **57** 1855–1858.
- Eschbach JW & Rivaldo JA 1962 Metastatic carcinoid; a familial occurrence. *Annals of Internal Medicine* **57** 647–650.
- Fujii T, Kawai T, Saito K, Hishima T, Hayashi Y, Imura J, Hironaka M, Hosoya Y, Koike M & Fukayama M 1999 MEN1 gene mutations in sporadic neuroendocrine tumors of foregut derivation. *Pathology International* **49** 968–973.
- Gimm O, Armanios M, Dziema H, Neumann HP & Eng C 2000 Somatic and occult germ-line mutations in SDHD, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. *Cancer Research* **60** 6822–6825.
- Goebel SU, Heppner C, Burns AL, Marx SJ, Spiegel AM, Zhuang Z, Lubensky IA, Gibril F, Jensen RT & Serrano J 2000 Genotype/phenotype correlation of multiple endocrine neoplasia type 1 gene mutations in sporadic gastrinomas. *Journal of Clinical Endocrinology and Metabolism* **85** 116–123.
- Gortz B, Roth J, Krahenmann A, de Krijger RR, Muletta-Feurer S, Rutimann K, Saremaslani P, Speel EJ, Heitz PU & Komminoth P 1999 Mutations and allelic deletions of the MEN1 gene are associated with a subset of sporadic endocrine pancreatic and neuroendocrine tumors and not restricted to foregut neoplasms. *American Journal of Pathology* **154** 429–436.
- Griffiths DF, Williams GT & Williams ED 1987 Duodenal carcinoid tumours, pheochromocytoma and neurofibromatosis: islet cell tumour, pheochromocytoma and the von Hippel–Lindau complex: two distinctive neuroendocrine syndromes. *Quarterly Journal of Medicine* **64** 769–782.
- Guo SS, Arora C, Shimoide AT & Sawicki MP 2002 Frequent deletion of chromosome 3 in malignant sporadic pancreatic endocrine tumors. *Molecular and Cellular Endocrinology* **190** 109–114.
- Guru SC, Goldsmith PK, Burns AL, Marx SJ, Spiegel AM, Collins FS & Chandrasekharappa SC 1998 Menin, the product of the MEN1 gene, is a nuclear protein. *PNAS* **95** 1630–1634.
- Han HS, Kim HS, Woo DK, Kim WH & Kim YI 2000 Loss of heterozygosity in gastric neuroendocrine tumor. *Anticancer Research* **20** 2849–2854.
- Harbour JW, Lai SL, Whang-Peng J, Gazdar AF, Minna JD & Kaye FJ 1988 Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science* **241** 353–357.
- Heppner C, Bilimoria KY, Agarwal SK, Kester M, Whitty LJ, Guru SC, Chandrasekharappa SC, Collins FS, Spiegel AM, Marx SJ & Burns AL 2001 The tumor suppressor protein menin interacts with NF-kappaB proteins and inhibits NF-kappaB-mediated transactivation. *Oncogene* **20** 4917–4925.
- Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarr JR, Linehan WM *et al.* 1994 Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *PNAS* **91** 9700–9704.
- Hessman O, Lindberg D, Skogseid B, Carling T, Hellman P, Rastad J, Akerstrom G & Westin G 1998 Mutation of the multiple endocrine neoplasia type 1 gene in nonfamilial, malignant tumors of the endocrine pancreas. *Cancer Research* **58** 377–379.
- Hessman O, Skogseid B, Westin G & Akerstrom G 2001 Multiple allelic deletions and intratumoral genetic heterogeneity in men1 pancreatic tumors. *Journal of Clinical Endocrinology and Metabolism* **86** 1355–1361.
- Hiyama K, Hasegawa K, Ishioka S, Takahashi N & Yamakido M 1993 An atypical carcinoid tumor of the lung with mutations in the p53 gene and the retinoblastoma gene. *Chest* **104** 1606–1607.
- Jakovovitz O, Nass D, DeMarco L, Barbosa AJ, Simoni FB, Rechavi G & Friedman E 1996 Carcinoid tumors frequently display genetic abnormalities involving chromosome 11. *Journal of Clinical Endocrinology and Metabolism* **81** 3164–3167.
- Jones PA & Baylin SB 2002 The fundamental role of epigenetic events in cancer. *Nature Reviews. Genetics* **3** 415–428.
- Kaji H, Canaff L, Lebrun JJ, Goltzman D & Hendy GN 2001 Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type beta signaling. *PNAS* **98** 3837–3842.
- Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F & Pinkel D 1992 Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* **258** 818–821.
- Kallioniemi OP, Kallioniemi A, Sudar D, Rutovitz D, Gray JW, Waldman F & Pinkel D 1993 Comparative genomic hybridization: a rapid new method for detecting and mapping DNA amplification in tumors. *Seminars in Cancer Biology* **4** 41–46.
- Kanthan R, Saxena A & Kanthan SC 2001 Goblet cell carcinoids of the appendix: immunophenotype and ultrastructural study. *Archives of Pathology and Laboratory Medicine* **125** 386–390.
- Kinova S, Duris I, Kovacova E, Stvrtina S, Galbavy S & Makaiova I 2001 Malignant carcinoid in two brothers. *Bratislavské Lekárske Listy* **102** 231–234.
- Kinzler KW & Vogelstein B 1997 Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386** 761–763.
- Knudson AG Jr 1971 Mutation and cancer: statistical study of retinoblastoma. *PNAS* **68** 820–823.
- Knudson AG Jr 1974 Heredity and human cancer. *American Journal of Pathology* **77** 77–84.
- Kovatch A, Friedland DM, Druck T, Hadaczek P, Huebner K, Comis RL, Hauck W & McCue PA 1998 Molecular alterations to human chromosome 3p loci in neuroendocrine lung tumors. *Cancer* **83** 1109–1117.
- Kytola S, Nord B, Elder EE, Carling T, Kjellman M, Cedermarck B, Juhlin C, Hoog A, Isola J & Larsson C 2002 Alterations of the SDHD gene locus in midgut carcinoids, Merkel cell carcinomas, pheochromocytomas, and abdominal paragangliomas. *Genes Chromosomes and Cancer* **34** 325–332.
- Le Douarin NM 1988 On the origin of pancreatic endocrine cells. *Cell* **53** 169–171.

- Lemmens IH, Forsberg L, Pannett AA, Meyen E, Piehl F, Turner JJ, Van de Ven WJ, Thakker RV, Larsson C & Kas K 2001 Menin interacts directly with the homeobox-containing protein Pem. *Biochemical and Biophysical Research Communications* **286** 426–431.
- Lengauer C, Kinzler KW & Vogelstein B 1997 Genetic instability in colorectal cancers. *Nature* **386** 623–627.
- Levin NA, Brzoska PM, Warnock ML, Gray JW & Christman MF 1995 Identification of novel regions of altered DNA copy number in small cell lung tumors. *Genes Chromosomes and Cancer* **13** 175–185.
- Lohmann DR, Fessler B, Putz B, Reich U, Bohm J, Prauer H, Wunsch PH & Hoffer H 1993a Infrequent mutations of the p53 gene in pulmonary carcinoid tumors. *Cancer Research* **53** 5797–5801.
- Lohmann DR, Funk A, Niedermeyer HP, Haupeil S & Hoffer H 1993b Identification of p53 gene mutations in gastrointestinal and pancreatic carcinoids by nonradioisotopic SSCA. *Virchows Archiv B, Cell Pathology including Molecular Pathology* **64** 293–296.
- Lollgen RM, Hessman O, Szabo E, Westin G & Akerstrom G 2001 Chromosome 18 deletions are common events in classical midgut carcinoid tumors. *International Journal of Cancer* **92** 812–815.
- Lopez-Egido J, Cunningham J, Berg M, Oberg K, Bongcam-Rudloff E & Gobl A 2002 Menin's interaction with glial fibrillary acidic protein and vimentin suggests a role for the intermediate filament network in regulating menin activity. *Experimental Cell Research* **278** 175–183.
- Mailman MD, Muscarella P, Schirmer WJ, Ellison EC, O'Dorisio TM & Prior TW 1999 Identification of MEN1 mutations in sporadic enteropancreatic neuroendocrine tumors by analysis of paraffin-embedded tissue. *Clinical Chemistry* **45** 29–34.
- Maitra A, Krueger JE, Tascilar M, Offerhaus GJ, Angeles-Angeles A, Klimstra DS, Hruban RH & Albores-Saavedra J 2000 Carcinoid tumors of the extrahepatic bile ducts: a study of seven cases. *American Journal of Surgical Pathology* **24** 1501–1510.
- du Manoir S, Speicher MR, Joos S, Schrock E, Popp S, Dohner H, Kovacs G, Robert-Nicoud M, Lichter P & Cremer T 1993 Detection of complete and partial chromosome gains and losses by comparative genomic *in situ* hybridization. *Human Genetics* **90** 590–610.
- Milunsky JM, Maher TA, Michels VV & Milunsky A 2001 Novel mutations and the emergence of a common mutation in the SDHD gene causing familial paraganglioma. *American Journal of Medical Genetics* **100** 311–314.
- Momparler RL & Bovenzi V 2000 DNA methylation and cancer. *Journal of Cellular Physiology* **183** 145–154.
- Momparler RL, Eliopoulos N & Ayoub J 2000 Evaluation of an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine, for the treatment of lung cancer and the future role of gene therapy. *Advances in Experimental Medicine and Biology* **465** 433–446.
- Nakano H, Yamamoto F, Neville C, Evans D, Mizuno T & Perucho M 1984 Isolation of transforming sequences of two human lung carcinomas: structural and functional analysis of the activated c-K-ras oncogenes. *PNAS* **81** 71–75.
- Oberg K 1998 Carcinoid tumors: current concepts in diagnosis and treatment. *Oncologist* **3** 339–345.
- Oberg K 2002 Carcinoid tumors: molecular genetics, tumor biology, and update of diagnosis and treatment. *Current Opinion in Oncology* **14** 38–45.
- Ohkura N, Kishi M, Tsukada T & Yamaguchi K 2001 Menin, a gene product responsible for multiple endocrine neoplasia type 1, interacts with the putative tumor metastasis suppressor nm23. *Biochemical and Biophysical Research Communications* **282** 1206–1210.
- Oliveira AM, Tazelaar HD, Wentzlaff KA, Kosugi NS, Hai N, Benson A, Miller DL & Yang P 2001 Familial pulmonary carcinoid tumors. *Cancer* **91** 2104–2109.
- Onuki N, Wistuba II, Travis WD, Virmani AK, Yashima K, Brambilla E, Hasleton P & Gazdar AF 1999 Genetic changes in the spectrum of neuroendocrine lung tumors. *Cancer* **85** 600–607.
- Pal T, Liede A, Mitchell M, Calender A & Narod SA 2001 Intestinal carcinoid tumours in a father and daughter. *Canadian Journal of Gastroenterology* **15** 405–409.
- Pannett AA & Thakker RV 1999 Multiple endocrine neoplasia type 1. *Endocrine-Related Cancer* **6** 449–473.
- Pannett AA & Thakker RV 2001 Somatic mutations in MEN type 1 tumors, consistent with the Knudson 'two-hit' hypothesis. *Journal of Clinical Endocrinology and Metabolism* **86** 4371–4374.
- Perren A, Barghorn A, Schmid S, Saremaslani P, Roth J, Heitz PU & Komminoth P 2001 The role of succinate-dehydrogenase-d (SDHD) tumor suppressor gene on 11q23 in endocrine tumors. *Virchows Archiv B, Cell Pathology including Molecular Pathology* **439** 389.
- Petersen I, Bujard M, Petersen S, Wolf G, Goeze A, Schwendel A, Langreck H, Gellert K, Reichel M, Just K, du Manoir S, Cremer T, Dietel M & Ried T 1997 Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung. *Cancer Research* **57** 2331–2335.
- Ramnani DM, Wistuba II, Behrens C, Gazdar AF, Sobin LH & Albores-Saavedra J 1999 K-ras and p53 mutations in the pathogenesis of classical and goblet cell carcinoids of the appendix. *Cancer* **86** 14–21.
- Rindi G, Luinetti O, Cornaggia M, Capella C & Solcia E 1993 Three subtypes of gastric argyrophil carcinoid and the gastric neuroendocrine carcinoma: a clinicopathologic study. *Gastroenterology* **104** 994–1006.
- Robertson KD & Jones PA 2000 DNA methylation: past, present and future directions. *Carcinogenesis* **21** 461–467.
- Sameshima Y, Matsuno Y, Hirohashi S, Shimosato Y, Mizoguchi H, Sugimura T, Terada M & Yokota J 1992 Alterations of the p53 gene are common and critical events for the maintenance of malignant phenotypes in small-cell lung carcinoma. *Oncogene* **7** 451–457.
- Schwartzmann G, Schunemann H, Gorini CN, Filho AF, Garbino C, Sabini G, Muse I, DiLeone L & Mans DR 2000 A phase I trial of cisplatin plus decitabine, a new DNA-hypomethylating agent, in patients with advanced solid tumors and a follow-up early phase II evaluation in patients with inoperable non-small cell lung cancer. *Investigational New Drugs* **18** 83–91.
- Shan L, Nakamura M, Nakamura Y, Utsunomiya H, Shou N, Jiang X, Jing X, Yokoi T & Kakudo K 1998 Somatic mutations in the RET protooncogene in Japanese and Chinese sporadic medullary thyroid carcinomas. *Japanese Journal of Cancer Research* **89** 883–886.
- Shimizu T, Tanaka S, Haruma K, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G & Shimamoto F 2000 Growth characteristics of rectal carcinoid tumors. *Oncology* **59** 229–237.
- Shivapurkar N, Toyooka S, Eby MT, Huang CX, Sathyanarayana UG, Cunningham HT, Reddy JL, Brambilla E, Takahashi T,

- Minna JD, Chaudhary PM & Gazdar AF 2002 Differential inactivation of caspase-8 in lung cancers. *Cancer Biology and Therapy* **1** 65–169.
- Sowa H, Kaji H, Canaff L, Hendy GN, Tsukamoto T, Yamaguchi T, Miyazono K, Sugimoto T & Chihara K 2003 Inactivation of menin, the product of the multiple endocrine neoplasia type 1 gene, inhibits the commitment of multipotential mesenchymal stem cells into the osteoblast lineage. *Journal of Biological Chemistry* **278** 21058–21069.
- Sukhodolets KE, Hickman AB, Agarwal SK, Sukhodolets MV, Obungu VH, Novotny EA, Crabtree JS, Chandrasekharappa SC, Collins FS, Spiegel AM, Burns AL & Marx SJ 2003 The 32-kilodalton subunit of replication protein A interacts with menin, the product of the MEN1 tumor suppressor gene. *Molecular and Cellular Biology* **23** 493–509.
- Tang X, Khuri FR, Lee JJ, Kemp BL, Liu D, Hong WK & Mao L 2000 Hypermethylation of the death-associated protein (DAP) kinase promoter and aggressiveness in stage I non-small-cell lung cancer. *Journal of the National Cancer Institute* **92** 1511–1516.
- Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, van Der Mey AG, van Ommen GJ, Cornelisse CJ & Devilee P 2001 Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. *Genes Chromosomes and Cancer* **31** 274–281.
- Teh BT 1998 Thymic carcinoids in multiple endocrine neoplasia type 1. *Journal of Internal Medicine* **243** 501–504.
- Teh BT, Zedenius J, Kytola S, Skogseid B, Trotter J, Choplin H, Twigg S, Farnebo F, Giraud S, Cameron D, Robinson B, Calender A, Larsson C & Salmela P 1998 Thymic carcinoids in multiple endocrine neoplasia type 1. *Annals of Surgery* **228** 99–105.
- Thakker RV 1993 The molecular genetics of the multiple endocrine neoplasia syndromes. *Clinical Endocrinology* **38** 1–14.
- Thakker RV 1994 The role of molecular genetics in screening for multiple endocrine neoplasia type 1. *Endocrinology and Metabolism Clinics of North America* **23** 117–135.
- Thakker RV 2000 Multiple endocrine neoplasia type 1. *Endocrinology and Metabolism Clinics of North America* **29** 541–567.
- Thakker RV & Ponder BA 1988 Multiple endocrine neoplasia. *Bailliere's Clinical Endocrinology and Metabolism* **2** 1031–1067.
- The European Consortium on MEN1 1997 Identification of the multiple endocrine neoplasia type 1 (MEN1) gene. *Human Molecular Genetics* **6** 1177–1183.
- Toliat MR, Berger W, Ropers HH, Neuhaus P & Wiedenmann B 1997 Mutations in the MEN I gene in sporadic neuroendocrine tumours of gastroenteropancreatic system. *Lancet* **350** 1223.
- Tonnies H, Toliat MR, Ramel C, Pape UF, Neitzel H, Berger W & Wiedenmann B 2001 Analysis of sporadic neuroendocrine tumours of the enteropancreatic system by comparative genomic hybridisation. *Gut* **48** 536–541.
- Toyooka S, Toyooka KO, Maruyama R, Virmani AK, Girard L, Miyajima K, Harada K, Ariyoshi Y, Takahashi T, Sugio K, Brambilla E, Gilcrease M, Minna JD & Gazdar AF 2001 DNA methylation profiles of lung tumors. *Molecular Cancer Therapeutics* **1** 61–67.
- Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, Edwards CR, Heath DA, Jackson CE, Jansen S, Lips K, Monson JP, O'Halloran D, Sampson J, Shalet SM, Wheeler MH, Zink A & Thakker RV 1996 Clinical studies of multiple endocrine neoplasia type 1 (MEN1). *Quarterly Journal of Medicine* **89** 653–669.
- Turner JJ, Leotlela PD, Pannett AA, Forbes SA, Bassett JH, Harding B, Christie PT, Bowen-Jones D, Ellard S, Hattersley A, Jackson CE, Pope R, Quarrell OW, Trembath R & Thakker RV 2002 Frequent occurrence of an intron 4 mutation in multiple endocrine neoplasia type 1. *Journal of Clinical Endocrinology and Metabolism* **87** 2688–2693.
- Tycko B 2000 Epigenetic gene silencing in cancer. *Journal of Clinical Investigation* **105** 401–407.
- Ullmann R, Petzmann S, Klemen H, Fraire AE, Hasleton P & Popper HH 2002 The position of pulmonary carcinoids within the spectrum of neuroendocrine tumors of the lung and other tissues. *Genes Chromosomes and Cancer* **34** 78–85.
- Vinik AI 2001 Carinoid tumours. In *Endocrinology*, edn. 4, pp 2533–2546. Eds LJ DeGroot & JL Jameson. Philadelphia: W.B. Saunders Company.
- Virmani AK, Rathi A, Sathyanarayana UG, Padar A, Huang CX, Cunningham HT, Farinas AJ, Milchgrub S, Euhus DM, Gilcrease M, Herman J, Minna JD & Gazdar AF 2001 Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. *Clinical Cancer Research* **7** 1998–2004.
- Vogelstein B & Kinzler KW 1993 The multistep nature of cancer. *Trends in Genetics* **9** 138–141.
- Wang EH, Ebrahimi SA, Wu AY, Kashefi C, Passaro E Jr & Sawicki MP 1998 Mutation of the MEN1 gene in sporadic pancreatic endocrine tumors. *Cancer Research* **58** 4417–4420.
- Yaguchi H, Ohkura N, Tsukada T & Yamaguchi K 2002 Menin, the multiple endocrine neoplasia type 1 gene product, exhibits GTP-hydrolyzing activity in the presence of the tumor metastasis suppressor nm23. *Journal of Biological Chemistry* **277** 38197–38204.
- Younossian AB, Bründler M & Tötsch M 2002 Feasibility of the new WHO classification of pulmonary neuroendocrine tumours. *Swiss Medical Weekly* **132** 535–540.
- Yumita W, Ikeo Y, Yamauchi K, Sakurai A & Hashizume K 2003 Suppression of insulin-induced AP-1 transactivation by menin accompanies inhibition of c-Fos induction. *International Journal of Cancer* **103** 738–744.
- Zhao J, de Krijger RR, Meier D, Speel EJ, Saremaslani P, Muletta-Feurer S, Matter C, Roth J, Heitz PU & Komminoth P 2000 Genomic alterations in well-differentiated gastrointestinal and bronchial neuroendocrine tumors (carcinoids): marked differences indicating diversity in molecular pathogenesis. *American Journal of Pathology* **157** 1431–1438.
- Zhuang Z, Ezzat SZ, Vortmeyer AO, Weil R, Oldfield EH, Park WS, Pack S, Huang S, Agarwal SK, Guru SC, Manickam P, Debelenko LV, Kester MB, Olufemi SE, Heppner C, Crabtree JS, Burns AL, Spiegel AM, Marx SJ, Chandrasekharappa SC, Collins FS, Emmert-Buck MR, Liotta LA, Asa SL & Lubensky IA 1997a Mutations of the MEN1 tumor suppressor gene in pituitary tumors. *Cancer Research* **57** 5446–5451.
- Zhuang Z, Vortmeyer AO, Pack S, Huang S, Pham TA, Wang C, Park WS, Agarwal SK, Debelenko LV, Kester M, Giru SC, Manickam P, Olufemi SE, Yu F, Heppner C, Crabtree JS, Skarulis MC, Venzon DJ, Emmert-Buck MR, Spiegel AM, Chandrasekharappa SC, Collins FS, Burns AL, Marx SJ, Lubensky IA *et al.* 1997b Somatic mutations of the MEN1 tumor suppressor gene in sporadic gastrinomas and insulinomas. *Cancer Research* **57** 4682–4686.