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RESEARCH

Molecular prognostic factors in small-intestinal neuroendocrine tumours

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Abstract

Background: Small-intestinal neuroendocrine tumours (SI-NETs) represent a heterogeneous group of rare tumours. In recent years, basic research in SI-NETs has attempted to unravel the molecular events underlying SI-NET tumorigenesis.
Aim: We aim to provide an overview of the current literature regarding prognostic and predictive molecular factors in patients with SI-NETs.
Method: A PubMed search was conducted on (epi)genetic prognostic factors in SI-NETs from 2000 until 2019.
Results: The search yielded 1522 articles of which 20 reviews and 35 original studies were selected for further evaluation. SI-NETs are mutationally quiet tumours with a different genetic make-up compared to pancreatic NETs. Loss of heterozygosity at chromosome 18 is the most frequent genomic aberration (44–100%) followed by mutations of *CDKN1B* in 8%. Prognostic analyses were performed in 16 studies, of which 8 found a significant (epi)genetic association for survival or progression. Loss of heterozygosity at chromosome 18, gains of chromosome 4, 5, 7, 14 and 20p, copy gain of the *SRC* gene and low expression of RASSF1A and P16 were associated with poorer survival. In comparison with genetic mutations, epigenetic alterations are significantly more common in SI-NETs and may represent more promising targets in the treatment of SI-NETs.
Conclusion: SI-NETs are mutationally silent tumours. No biomarkers have been identified yet that can easily be adopted into current clinical decision making. SI-NETs may represent a heterogeneous disease and larger international studies are warranted to translate molecular findings into precision oncology.

Key Words
- small intestinal
- neuroendocrine tumours
- carcinoid
- genetics
- epigenetics
- prognosis
- survival

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Introduction

Well-differentiated neuroendocrine tumours (NETs) represent a heterogeneous group of rare tumours, which have a relatively indolent disease course. Primary NETs can arise from neuroendocrine cells at various anatomic sites. They most commonly develop in the gastrointestinal tract and bronchopulmonary system (1, 2, 3). NETs can be classified as functional or non-functional, based on whether they cause clinical symptoms as a result of hormone secretion or not. In patients with metastatic small-intestinal NETs (SI-NETs), the carcinoid syndrome is common, which is characterised by diarrhoea, episodic flushing, bronchospasm and often carcinoid heart disease leading to right valvular dysfunction (4). Patients with non-functional SI-NETs are often asymptomatic or experience non-specific symptoms resulting in metastatic disease at the time of diagnosis in 27–73% of patients (1, 2, 3). In contrast to pancreatic NETs, SI-NETs are not known to arise in the context of hereditary syndromes,

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for example multiple endocrine neoplasia (MEN) type 1 or 2 and Von Hippel Lindau disease.

The reported incidence of SI-NETs has increased over the last four decades, from 0.2 per 100,000 individuals in 1973 to 1.25 per 100,000 individuals in 2012 (5). This progressive rise can mainly be contributed to more frequent use and improvements of diagnostic modalities or alterations in pathological disease definition (2, 5). In the group of gastroenteropancreatic NETs, SI-NETs are second most prevalent after rectum NETs and followed by pancreatic NETs (5). Moreover, SI-NETs are the most frequent cancer type of the small intestine (6).

Currently, treatment for patients with SI-NETs is based on the availability of several treatment modalities, for example, surgery, liver-directed therapies, somatostatin receptor analogues and peptide receptor radionuclide therapy rather than on precision medicine. In case of non-functional, advanced and progressive SI-NETs, everolimus, targeting the *P13K/AKT/mTOR* (mammalian target of rapamycin) pathway, has demonstrated anti-proliferative effects (7, 8, 9). However, there is no biomarker available that predicts response to everolimus.

To conclude, personalised treatment based on molecular profiling has not yet entered the arena of treatment modalities in advanced SI-NETs.

In order to move towards precision medicine, the genomic landscape of SI-NETs has been under increasing investigation over the past years in the hope of unravelling the molecular events underlying NET tumorigenesis, facilitating the identification of novel therapeutic targets, rational (targeted) therapy management strategies and to improve prognosis. Recently, whole-genome sequencing of primary pancreatic NETs revealed several genomic events which characterise their pathogenesis and are associated with tumour progression (10). In general, gene expression-based subtyping has led to new classifications of multiple tumour types. In contrast, the genomic landscape of SI-NETs remains poorly elucidated and biomarkers have not yet been identified. Moreover, the genetic constitution of SI-NETs has been shown to differ compared to pancreatic NETs (11). With this review we aim to provide the clinician treating SI-NETs with an overview of the recent studies evaluating molecular characteristics of SI-NETs and their predictive and prognostic significance.

Methods

A literature search was performed in PubMed in March 2019. As our main objective was to provide an up to date overview of the current literature regarding prognostic molecular factors in SI-NETs for clinicians treating patients with SI-NETs, we did not aim to perform a formal systematic review. The domain of this search consisted of adult patients with sporadic SI-NETs, the determinant of genetic or epigenetic alterations and the outcomes of prognosis, survival or progression. Synonyms of SI-NETs and (epi)genetic alterations with the outcome described as prognosis, survival and progression were used for the search. Search terms and syntax are described in detail in Table 1. Screening based on title and abstract was conducted by one reviewer, in case of uncertainties a second reviewer was consulted. Citation search of the included articles was performed to identify additional original studies.

Inclusion criteria consisted of patient populations >18 years, human, full-text available in English, published between 01/01/2000 and 01/03/2019 and studies on gastroenteropancreatic NETs. Studies with a patient population with underlying genetic syndromes, no separate genome analysis for SI-NETs, using previously published results and on the taxonomy of SI-NETs were excluded.

Results

The PubMed search yielded 1522 hits, of which 1461 articles were excluded after screening of title and abstract (Fig. 1). Following the full-text screening of 61 articles,

Table 1 Search terms and syntax.

Syntax in PubMed	((((((carcinoid[Title/Abstract]) OR ((((((tumor*[Title/Abstract]) OR tumour*[Title/Abstract]) OR neoplas*[Title/Abstract]) OR malignan*[Title/Abstract])) AND ((neuroendocrin*[Title/Abstract]) OR ((((((small[Title/Abstract] AND bowel[Title/Abstract])) OR ileal*[Title/Abstract]) OR jejun*[Title/Abstract]) OR duoden*[Title/Abstract]) OR midgut[Title/Abstract])))))) AND ((((((((((genom*[Title/Abstract]) OR epigenetic*[Title/Abstract]) OR gene*[Title/Abstract]) OR exom*[Title/Abstract]) OR chromosom*[Title/Abstract]) OR molecular*[Title/Abstract]) OR allel*[Title/Abstract])) OR sequenc*[Title/Abstract]) OR (((((methylation*[Title/Abstract]) OR mutation*[Title/Abstract]) OR alteration*[Title/Abstract]) OR amplificat*[Title/Abstract]) OR loss[Title/Abstract]))))) AND (((prognos*[Title/Abstract]) OR survival*[Title/Abstract]) OR progressi*[Title/Abstract]))
Search terms	'carcinoid', 'tumor', 'tumour', 'neoplasia', 'malignan*', 'neuroendocrin*', 'small bowel', 'ileal', 'jejun*', 'duoden*', 'midgut', 'genom', 'epigenetic*', 'gene*', 'exom*', 'chromosom*', 'molecular*', 'allel*', 'sequenc*', 'methylation*', 'mutation*', 'alteration*', 'amplificat*', 'loss', 'prognos*', 'survival', 'progressi*'

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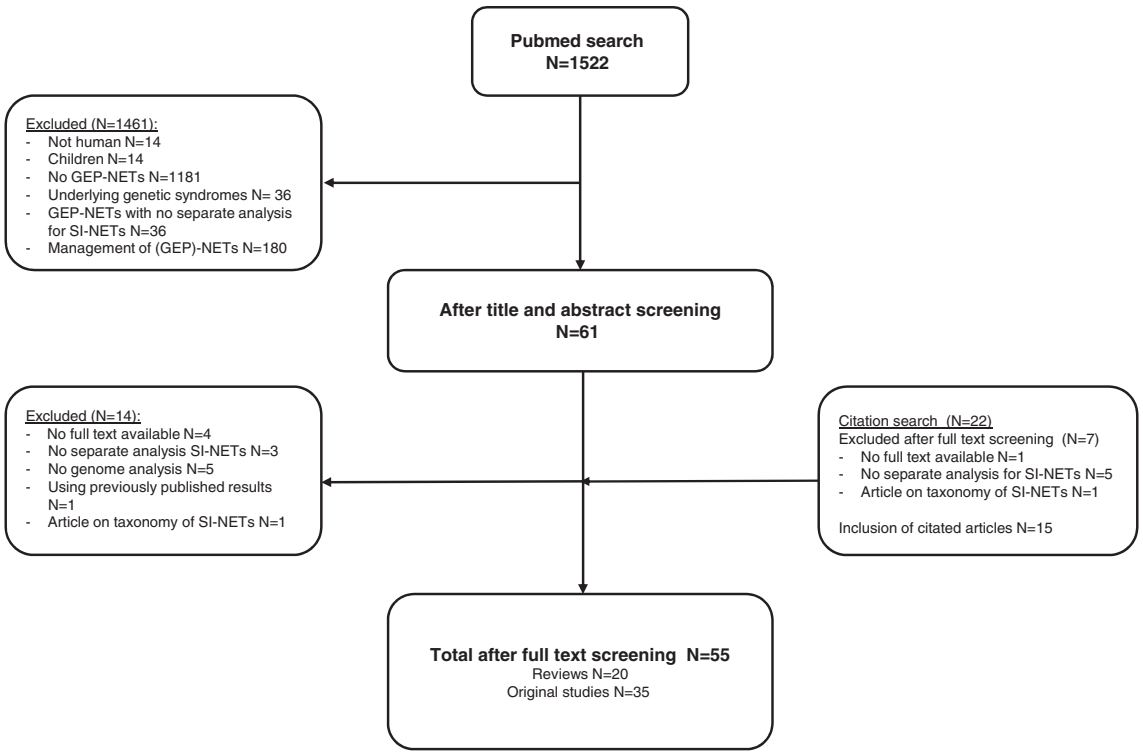

Figure 1
Flowchart of search and screening process in PubMed.

14 articles were excluded. The citation search identified 22 additional articles of which 7 were excluded. In total 55 relevant articles were found, consisting of 20 reviews and 35 original studies. The results of the selected original studies are shown in Table 2. Our review will discuss the most relevant studies, with a special focus on the prognostic implications of the identified molecular alterations. The identified studies describe different genomic events and altered expression of several proteins which play a key role in various molecular pathways involved in SI-NET tumorigenesis. Events which have been described in multiple studies and are discussed in this paper are shown in Fig. 2.

Genetics of SI-NETs

Chromosomal aberrations

From genomic profile studies, two different groups of SI-NETs can be identified, one which is characterised by loss of heterozygosity (LOH) of chromosome 18 as an early event and the other group which has no alterations of chromosome 18 and shows clustered gains on chromosomes 4, 5, 7, 14 and 20 (11, 12, 13, 14, 15).

Multiple studies reported loss of one copy of chromosome 18, with an incidence of 44–100% in primary SI-NETs (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22). Chromosome 18 harbours several candidate tumour suppressor genes, including *DCC* (deleted in colorectal cancer; involved in axon guidance), *SMAD4* (mothers against decapentaplegic homolog 4; *TGFB* signal transduction), *SMAD2* (mothers against decapentaplegic homolog 2; *TGFB* signal transduction) and *TCEB3C* (transcriptional elongation factor B polypeptide 3C; encoding Elongin A3; RNA transcription). Banck *et al.*, who performed whole-exome sequencing (WES) on 48 well-differentiated SI-NETs, found *SMAD2* and *SMAD4* monoallelic deletions in 21 tumours (23, 24). Edfeldt *et al.* ($n=43$) identified that in the majority of SI-NETs decreased expression of Elongin A3 (77%) was present and that the remaining *TCEB3C* gene was epigenetically silenced by DNA hypermethylation (25).

Nieser *et al.* ($n=148$) performed the first comprehensive study to identify chromosome 18 related events at genetic, epigenetic and gene/protein expression level, which only found *DCC* to be affected by the monoallelic loss of chromosome 18 (22). In addition, Simbolo *et al.* ($n=52$) observed copy loss of multiple genes located on chromosome 18: *CDH19* (cadherin 19; cell adhesion; 46.2%), *BLC2* (B-cell-lymphoma; regulation of cell death; 42.3%), *DCC* (42.3%) and *SMAD4* (28.8%) (15).

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Table 2 Overview of studies.

Study	Publication year	No. of patients	Domain	Analysis technique	Molecular aberrations	Prognostic association	Remarks
Löllgen *et al.*	2001	8	Metastatic midgut NETs (6 ileal, 1 ileocecal valve, 1 ascending colon)	Genome-wide LOH* screening with microsatellite markers	Deletions on Chr* 18 in 88% of midgut NETs	–	Analysis included 1 colon NET
Wang *et al.*	2005	47	Ileal NETs ($n = 16$)	Microsatellite markers, PCR amplification, sequencing of the *BRAF* gene	Allelic loss of both arms of chromosome 18 in 69% No *BRAF* mutations were identified	–	
Zhang *et al.*	2006	33	SI-NETs with matched primary and metastatic tumours	Methylation-specific PCR, Western blot and immunochemistry	Methylation of *RASSF1A* and *CTNNB1* promoters more frequent in metastatic vs primary tumours ($P = 0.013$ and $P = 0.004$, respectively)	–	–
Kim *et al.*	2007	29	Well-differentiated ileal NETs ($n = 15$)	Genome-wide high-density single-nucleotide polymorphism array analysis	Loss of Chr 18 in 67%, loss of Chr 21 or 21q in 13%	No correlation between loss of Chr 18 and 21 with survival	–
Choi *et al.*	2007	35	Ileal NETs ($n = 15$)	Pyrosequencing	Hypomethylation of LINE-1 was greater in ileal NETs than in non-ileal and pNETs ($P = 0{,}047$), and tumours with lymph node metastasis ($P = 0.02$), Chr 18 loss ($P = 0.001$) and RAS-association domain family I, isoform A gene methylation ($P = 0.02$).	No survival differences were observed based on LINE-1 methylation levels	–
Kulke *et al.*	2008	18	Primary and metastatic SI-NETs ($n = 24$)	High resolution arrays of single-nucleotide polymorphisms	Loss of Chr 18 in 61%, Chr 9 in 33% and Chr 16 in 22%. Gains on Chr 4 (33%) and Chr 5,7,20 and 14q (17%)	–	–
Andersson *et al.*	2009	43	Ileal NETs	High-resolution array based on comparative genomic hybridisation	Loss of Chr 18 in 74%. Other frequent copy number alterations were gain of Chr 4 (30%), 5 (28%), 14 (23%) and 20 (33%), and loss of 11q22.1-q22.2, 11q22.3-q23.1 and 11q23.3 (21%) and loss of 16q12.2-q22.1 and 16q23.2-qter (16%)	Gain of Chr 14 was a strong predictor of poor survival ($P < 0.001$; HR 8.39 (95% CI: 3.04–23.11)). Loss of 3p13 resulted in a reduced risk of death ($P = 0.028$; HR 0.14 (95% CI: 0.02–0.8)). Significant correlation between gain of Chr 7, 14 and 20, and loss of 18 and overall survival ($P < 0.05$)	–
Cunningham *et al.*	2010	45 (37 sporadic and 8 familial)	Sporadic and familial ileal NETs (61 tumour samples)	High-resolution genomic and gene expression profiling	Chr 18 aberrations in both sporadic and familial ileal NETs (100 vs 38%) Frequent gain of Chr 7 in metastasis vs primary tumour (16 vs 0%)	No difference in overall survival in patients with or without a gain of Chr 14	–

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Ruebel *et al.*	2010	8	Primary and metastatic ileal NETs	RT-PCR, miRNA expression assay, Northern blotting, *in situ* hybridisation	Downregulation of miRNA-133a (ratio 0.27*), –145 (ratio 0.33*), –146 (ratio 0.36*), –222 (ratio 0.41*) and –10b (ratio 0.44*) in 100% of primary vs matching metastasis, upregulation of miRNA-183 (ratio 1.99*), –488 (ratio 1.56*), and –19a+b (ratio 1.31*) in 75% metastatic ileal NETs compared to primary tumours	-	*Mean metastatic/ primary tumours ratio <1 (upregulated in primary tumours) *Mean metastatic/ primary ratio tumours ratio >1 (downregulated in primary tumours)
Walsh *et al.*	2011	239 cases and 110 controls	Ileal NETs	Genome-wide association study single-nucleotide polymorphism genotyping	No single-nucleotide variants significantly associated with ileal NETs, rs2208059 in *KIF16B* had a trend towards statistical significance 14/226 cases (6.19%) and 2/97 controls (2.06%) heterozygous copy number deletions at 18q22.1.	–	–
Edfeldt *et al*	2011	19	SI-NETs ($n = 18$), lymph node metastases ($n = 17$), liver metastases ($n = 7$)	Gene expression arrays, qPCR	Three clusters of gene expression profiles were identified distinguishing primary tumours (11/18) from lymph node metastases (5/17) and a third group consisting of liver metastases (7/7), lymph node metastases (12/17) and primary tumours (7/8). The different profiles suggest changes in the development from primary tumour to metastases	No association was found between group 1 and group 3 for indolent or progressive disease course ($P = 0.15$)	–
Stricker *et al.*	2012	58	SI-NETs ($n = 17$)	Pyrosequencing	LINE1 hypomethylation was detected in 82% of SI-NETs	–	–
Banck *et al.*	2013	48	SI-NETs	Exome sequencing	0.1 SNVs* per 10^6 nucleotides No recurrent mutations in cancer genes. 197 protein-altering SNVs affected multiple cancer genes, including *FGFR2*, *MEN1*, *HOOK3*, *EZH2*, *MLF1*, *CARD11*, *VHL*, *NONO* and *SMAD1*. Mutually exclusive amplification of *AKT1* or *AKT2* was the most common event in 16 patients with alteration of *P13K/Akt/mTOR* signalling	–	–
Francis *et al.*	2013	180, including 48 from Banck *et al.*	SI-NETs	Exome and genome sequencing	Frameshift mutations of *CDKN1B* in 8% SI-NETs (8%; 95% CI 4.7-12.7), hemizygous deletions encompassing *CDKN1B* in 14%	–	–

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Table 2 Continued

Study	Publication year	No. of patients	Domain	Analysis technique	Molecular aberrations	Prognostic association	Remarks
Li *et al.*	2013	24	SI-NETs (5 primary tumours, 5 mesentery metastasis, 5 liver metastasis)	Affymetrix Genechip miRNA array, qRT-PCR, Northern blot Analysis	miRNA-96 ($P < 0.01$ compared to mesentery metastasis (MM) and $P < 0.001$ liver metastases (LM)), –182 (MM $P < 0.05$, LM $P < 0.001$), –183 (MM $P < 0.001$, LM $P < 0.01$), –196a (MM $P < 0.001$, LM $P < 0.001$) were upregulated during tumor progression miRNA-31 (MM $P < 0.05$, LM < 0.05), –129-5p (MM $P < 0.01$, LM $P < 0.001$), –133a (MM $P < 0.05$, LM <0.05) and –215 (MM $P < 0.05$, LM $P < 0.05$) were downregulated	–	–
Hashemi *et al.*	2013	30	SI-NETs ($n = 18$) and metastases ($n = 12$)	Comparative genome hybridisation, qPCR	Loss of chromosome 18 in 70%. Copy number losses on chromosome 11 (23%), 16 (20%), and 9 (20%), with regions of recurrent copy number loss identified in 11q23.1-qter, 16q12.2-qter, 9pter-p13.2 and 9p13.1-11.2. Gains detected in chromosomes 14 (43%), 20 (37%), 4 (27%), and 5 (23%) with recurrent regions of copy number gain in 14q11.2, 14q32.2-32.31, 20pter-p11.21, 20q11.1-11.21, 20q12-qter, 4 and 5. Differences between primary tumours and metastases; loss of 16q ($P = 0.003$) loss and gain of Chr 7 ($P = 0.016$).	Gain in 20pter-p11.21 was associated with short survival ($P = 0.013$). No other significant associations were observed between recurrent copy number alterations and survival	–
Bottarelli *et al.*	2013	30	Ileal NETs	DNA fragment analysis and sequencing of the mutation cluster region of the *APC* gene	*APC* gene mutations in 23%, of which missense (57%) and nonsense/frameshift (14%) mutations	No association was found with tumour progression	–
Edfeldt *et al.*	2013	43	SI-NETs	Gene copy number determination by PCR, real time quantitative RT-PCR, RNA interference, CpG methylation pyrosequencing	One copy deletion in 89% SI-NETs with reduced Elongin A3 expression in 77%.	–	–

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Fotouhi *et al.*	2014	33	SI-NETs (*n* = 44)	Pyrosequencing, ELISA-based quantification of global DNA methylation, qRT-PCR	Methylation was seen in *WIF1* (methylation index (MI) 50%, (16–92%)), *RASSFA1* (MI 16% (1–69%)), *CTNNB1* (MI 13% (4–34%)), *CXCL14* (MI 14% (3–39%)), *NKX2-3* (MI 10% (2–28%)), *P16* (*CDKN2A*) (MI 4% (1–33%)), *LAMA1* (MI 10% (4–24%), and *CDH1* (MI 8% (3–22%). *APC* (MI 3% (2–8%)), *CDH3* (MI 6% (3–12%)), *HIC1* (MI 5% (1–12%)), *P14* (*CDKN2A*) (MI 5% (2–17%)), *SMAD2* (MI 4% (1–8%)) and *SMAD4* (MI 3% (1–6%) had low levels of methylation. *WIF1* methylation was significantly increased (*P* = 0.001) and *WIF1* expression was reduced in SI-NETs vs normal references (*P* = 0.003). *WIF1*, *NKX2-3* and *CXCL14* expression was reduced in metastases vs primary tumours (*P* < 0.02). Global methylation of LINE1 was reduced in tumours vs normal references (65 vs 75%), and was associated with loss of Chr18p and 18q (*P* = 0.022, *P* = 0.003, respectively)	Low expression of RASSF1A and P16 were associated with poor survival (*P* = 0.045 and *P* = 0.011, respectively). Gene-specific promoter methylation or global methylation did not influence survival	
Verdugo *et al.*	2014	20	Matched primary SI-NETs (n = 10) and their mesenteric lymph node metastases (n = 10)	Human methylation 27 BeadChip array profiling	*RUNX3*, *TP73* and *CHFR* were highly methylated (β value ≥0.9). At Chr 18q21-qter (β value >0.7), *SETBP1*, *ELAC1*, *MBD1*, *MAPK4*, and *TCEB3C* were methylated including several members of the Serpin peptidase inhibitor family (*SERPINB3*, *SERPINB5*).	SI-NETs with a higher methylation index had a more aggressive phenotype	–
Norlen *et al.*	2014	15	Peritoneal carcinomatosis of SI-NETs (*n* = 8) and controls (*n* = 7)	Single-nucleotide polymorphism array	Two groups were identified, group A with a greater proportion of patients with PC (86%) than group B (25%), with LOH of the entire or major part of chromosome 18 in group A (75%) compared to limited LOH (75%) or no LOH (25%) in group B		–
Crona *et al.*	2015	200	SI-NETs (*n* = 362)	Automated Sanger sequencing of the *CDKN1B* gene, immunohistochemistry	Mutations of *CDKN1B* in 8.5%. Inter- and intratumour heterogeneity at the *CDKN1B* locus was present (33 and 11% respectively). Expression of p27 did not correlate with *CDKN1B* mutation status. No differences in clinical characteristics between *CDKN1B* mutated and *CDKN1B* wild-type tumour carriers were found	No correlation was found between survival and *CDKN1B* mutation status (HR 0.76; 95% CI 0.36–1.57)	–

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Table 2 Continued

Study	Publication year	No. of patients	Domain	Analysis technique	Molecular aberrations	Prognostic association	Remarks
Maxwell *et al.*	2015	90	SI-NETs	Exome sequencing and CNV analysis by quantitative PCR	*CDKN1B* frameshift mutations in 3.5% of SI-NETs (95% CI: 1.1–9.8%), 1 patient had a hemizygous deletion of *CDKN1B* and 2 patients duplications (3.4%; 95% CI 0.41–7.2%). Mutations of *CDKN1B* occurred in 6.9%	–	–
Delgado Verdugo *et al.*	2015	7	SI-NETs	Whole-exome capture, NGS*, high resolution SNP array, copy number variation analysis	Loss of Chr18 in 71% of SI-NETs. No tumour-specific somatic mutation was identified	–	
Bollard *et al.*	2015	38	Ileal NETs	Immunochemistry, methylation-specific PCR	*SEMA3F* expression was lost in 96% ileal NETs and all their metastases. *SEMA3F* loss of expression was associated with promoter gene methylation (no *P* value provided)	–	–
Karpathakis *et al.*	2015	97	SI-NETs	Whole-genome or targeted *CDKN1B* sequencing, Human methylation 450 BeadChip array profiling, methylated DNA immunoprecipitation sequencing, CNV analysis, whole-genome DASL* expression array profiling	Subgroup 1: chromosome 18 LOH, *CDKN1B* mutations, CIMP*, negativity. Subgroup 2: absence of arm-level CNVs, CIMP positivity Subgroup 3: multiple CNVs Epimutations were found at a recurrence rate up to 85%, and 21 epigenetically dysregulated genes were identified, including *CDX1* (86%), *CELSR* (84%), *FBP* (84%), and *GIPR* (84%).	3 subgroups of SI-NETs with different PFS* (not reached at 10 years vs 56 months vs 21 months; $P = 0.04$)	
Miller *et al.*	2016	90	Primary SI-NETs ($n = 28$), adjacent normal small bowel ($n = 14$), matched lymph node metastases ($n = 24$), normal lymph node metastases ($n = 7$), normal liver ($n = 2$) and liver metastasis ($n = 15$)	NanoString miRNA profiling, qRT-PCR, luciferase reporter assays and immunoblotting	miR-204-5p ($P = 2.44 \times 10^{-67}$), miR-7-5p ($P = 2.57 \times 10^{-144}$) and miR-375 ($P = 6.30 \times 10^{-67}$) were upregulated and miR-1 ($P = 0.0004$) and MiR-143-3 ($P = 8.11 \times 10^{-9}$) were downregulated in lymph node and liver metastases vs primary tumours	–	–
Andersson *et al.*	2016	33	Well-differentiated distal ileal NETs	Genome-wide sequencing	Loss of chromosome 18 in 65% and gains of chromosome 4,5,7,14 and 20 in 51%. Loss of *CDKN1B* in 8%. 3 subgroups were identified. The prostaglandin E receptor 2 (*PTGER2*) is the most activated in tumours of higher grade ($P = 4.4 \times 10^{-10}$), whereas Forkhead box M1 (*FOXM1*) was the most activated regulator in tumours with gain of chromosome 14 ($P = 2.5 \times 10^{-4}$)	The largest subgroup ($n = 17$) was characterised by longer survival ($P < 0.05$) and higher expression of neuroendocrine markers, including SSTR2. Tumours with higher grade (G2/3) or gain of chromosome 14 were associated with shorter patient survival ($P < 0.05$) and increased expression of cell cycle-promoting genes	Analysis included 1 ileal NEC

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Dumanski *et al.*	2017	239	Sporadic (215) and familial (24) SI-NETs compared to three control cohorts with 35,688 subjects	NGS* of exome or whole-genome DNA	A mutation in the *MUTYH* gene was significantly enriched in SI-NETs (both sporadic and familial) compared to controls (OR 5.09; 95% 1.56–14.74; $P = 0.0038$)	–	–
Karpathakis *et al.*	2017	20	SI-NETs and matched liver metastasis	Human methylation 450 BeadChip array profiling, methylated DNA immunoprecipitation sequencing, whole-genome DASL* expression array profiling	SI-NET liver metastasis show Chr18 LOH in 79%. Amplification of Chr20 (42%), deletion of Chr19 (34%) and gain of 17q (21%) in liver metastasis. In liver metastasis enrichment of multiple cancer-related pathways was seen; *P13K* signalling events, *ERBB1* downstream signalling, *PDGFRB* signalling pathway and *mTOR* pathway (adjusted $P < 0.001$). Using a previously defined panel of 21 epimutated genes, a trend of progressive dysregulation in liver metastasis compared to primary SI-NETs was observed	–	–
Shi *et al.*	2017	267	SI-NETs ($n = 55$)	Immunochemistry, *CDKN1B* sequencing	*CDKN1B* mutations in 10.9%. No clear association was found between *CDKN1B* mutation and protein expression	A trend towards shorter overall survival associated with low expression of *CDKN1B* was observed (multivariate hazards ratio, 2.04; 95% CI 1.06–3.93; $P = 0.03$). *CDKN1B* mutation was not associated with survival.	–
Nieser *et al.*	2017	148	SI-NETs	qRT-PCR, Western blot, immunochemistry, NGS*, SNP array analysis, miRNA analysis by qRT-PCR	Chr 18 LOH in 65%. Only *DCC* (deleted in colorectal cancer) revealed loss of/greatly reduced expression in 29%. No additional genetic or epigenetic alterations were present on Chr18	Loss of *CABLES* did not correlate with survival	–
Keck *et al.*	2018	12	Matched small bowel tissue, primary SI-NETs, liver metastases	RNA sequencing, Whole transcriptome microarrays, qPCR	Serial differential expression was validated in 7/10 genes, with several interacting members of the *AKT*, *MYC*, or *MAPK3* pathways. Liver metastases had underexpression of *PMP22* ($P < 0.001$) High expression of *SERPINA10* (primary $p < 0.001$, liver metastases <0.001) and *SYT13* (primary $P < 0.001$, liver metastases <0.001) was characteristic of primary SI-NETs and liver metastases	–	–

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Table 2 Continued

Study	Publication year	No. of patients	Domain	Analysis technique	Molecular aberrations	Prognostic association	Remarks
Simbolo *et al.*	2018	52	SI-NETs	High-coverage target sequencing, qPCR, FISH, expression analysis of *SRC* gene, immunohistochemistry	Mutations in *CDKN1B* (9.6%), *APC* and *CDKN2C* (each 7.7%), *BRAF*, *KRAS*, *PIK3CA* and *TP53* (each 3.8%). Frequent allelic loss of 4 genes located on Chr 18 (*BCL2*, *CDH19*, *DCC* and *SMAD4*) in 44.2% and losses on chromosomes 11 (38%) and 16 (15%). Gains on chromosomes 4 (31%), 5 (27%), 14 (36%), and 20 (20%).	*SRC* gene copy number gains were associated with a poorer prognosis ($P = 0.0047$)	
Yao *et al.*	2019	89	SI-NETs (small intestine, jejunum, ileum, duodenum, cecum, CUP)	Whole-exome and targeted sequencing	*BCOR* (5.6%) and *CDKN1B* (4.5%) most frequently mutated genes. LOH was present in approximately 50% and copy number gains of Chr 4,5,14 and 20 in >25%. Five distinct genomic clusters were identified (cluster 1: LOH of Chr 18, cluster 2: gain Chr 5 and 7, cluster 3: gain Chr 4,5,14 and 20, cluster 4: Chr 4,5,7,14 and 20, cluster 5: copy number gains across most chromosomes)	Lower generalised chromosomal instability in SI-NETs ($n = 55$) was associated with longer survival compared to high CIN ($n = 38$) with a PFS of 18.6 vs 9.2 months (HR 0.41; 95% CI 0.24-0.73; $P = 0.0021$)	Analysis included CUP and cecum NETs

*Chr, chromosome; CIMP, CpG island methylator phenotype; CUP, cancer of unknown primary; DASL, cDNA-mediated Annealing, Selection, extension and Ligation; GI-NET, gastrointestinal NET; LOH, loss of heterozygosity; NGS, next-generation sequencing; OR, odds ratio; PFS, progression-free survival; SNV, somatic single variants; WT, wildtype.

The clinical significance of LOH of chromosome 18 has been evaluated in multiple studies, either focussing solely on LOH of chromosome 18 or as part of a molecular profile study. According to Andersson *et al.* ($n = 43$) LOH of chromosome 18 is associated with worse overall survival (13). In contrast, Kim *et al.* did not find a significant correlation between loss of chromosome 18 and survival (18). Contrarily, Yao *et al.* ($n = 89$) found that SI-NETs with low generalised chromosomal instability (CIN) (which consisted of a cluster with LOH of chromosome 18) displayed significantly longer median PFS than those with a high CIN (which consisted of 3 clusters with different combinations of gains of chromosome 4, 5, 7, 14, 20 and 1 cluster with copy number gains across most chromosomes). PFS in patients with a low CIN ($n = 55$) was 18.6 vs 9.2 months in high CIN ($n = 38$) (HR; 0.41; 95% CI 0.24–0.73; $P = 0.0021$) (11).

As described by the clusters of Yao *et al.*, recurrent gains of chromosome 4, 5, 7, 14 and 20 are common in SI-NETs (11, 12, 13, 14, 15, 18, 19, 21). In two studies by Andersson *et al.*, gain of chromosome 14 was seen in 6 of 32 well-differentiated SI-NETs and was associated with higher tumour grade and shorter survival (HR 8.39; 95% CI 3.04–23.11) (13, 21). However, Cunningham *et al.* ($n = 45$) and Simbolo *et al.* ($n = 52$) could not corroborate these findings (15, 19). Hashemi *et al.* ($n = 30$) studied copy number alterations (CNAs; gains and losses of areas of the chromosome) and reported an association between gain of 20pter-p11.21 and worse survival (14), which was also not confirmed by the findings of Simbolo *et al.* (15). Generalised chromosomal instability seems to be a common feature of SI-NETs. This phenomenon could possibly be a reflection of diverse underlying defects in chromosomal maintenance that drive SI-NET development (11).

Mutational status

Banck *et al.* analysed 48 primary SI-NETs, predominantly grade 1, by massively parallel exome sequencing and detected a low mutation rate in the SI-NET genomes with an average of 0.1 somatic single-nucleotide variants (SNVs) per 10^6 nucleotides in the exome, suggesting that SI-NETs are mutationally quiet tumours (23). No recurrent mutations in the 215 sequenced target genes were found. In the studied SI-NETS, 197 protein-altering SNVs were identified, affecting a multitude of cancer genes including *FGFR2*, *MEN1*, *HOOK3*, *EZH2*, *MLF1*, *CARD11*, *VHL*, *NONO*, *FANCD2*, *SMAD1* and *BRAF*. In 29% of SI-NETs, there were genetic alterations in the *P13K/AKT/mTOR* pathway and mutually exclusive amplification of *AKT1* or *AKT2* were

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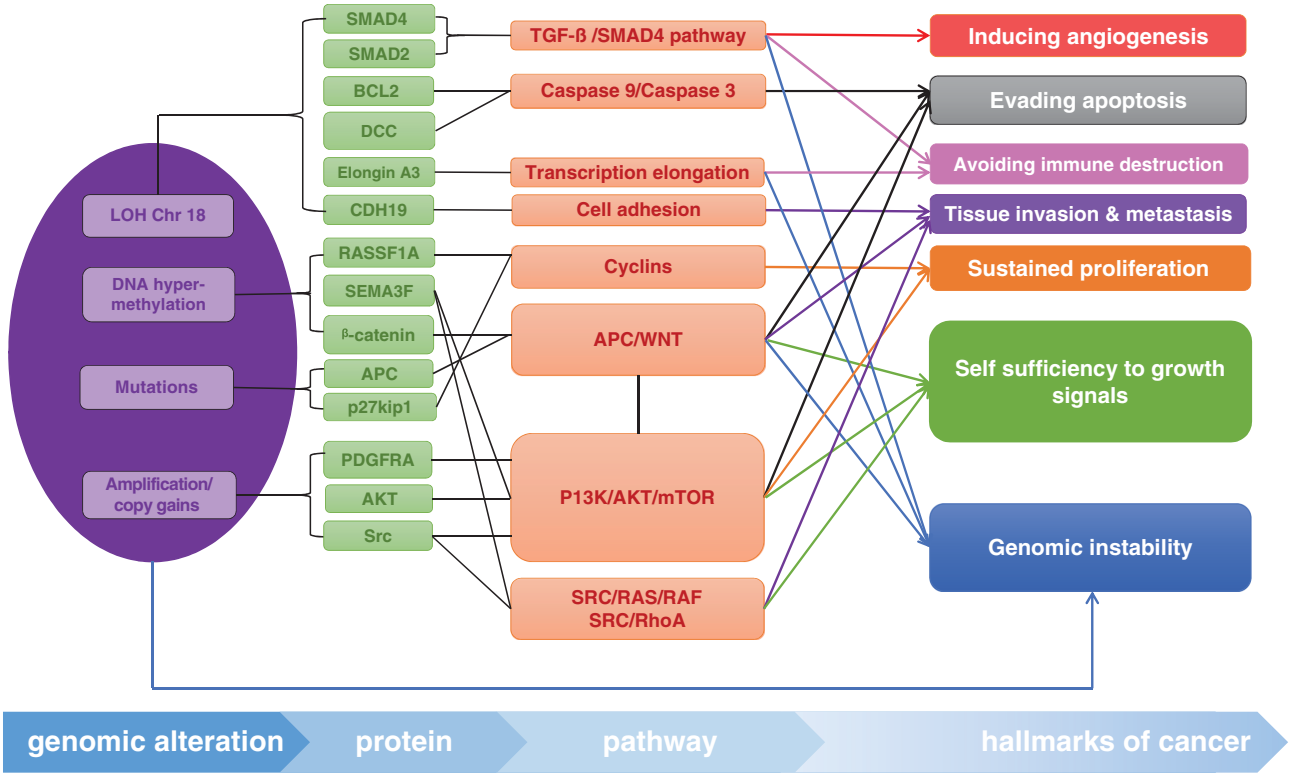

Figure 2
The studies presented in this review identified the deregulation of the expression of multiple genes in SI-NETs, which are commonly associated with carcinogenesis in other tumours. In the figure above, only those molecular alterations that have been found in multiple studies are depicted, together with their presumed role as key regulators of different cell functions and their possible effect on tumour progression as defined by the hallmarks of cancer (55).

common. Amplifications were also observed at the *PDFDR* (platelet-derived growth factor receptor alpha) locus in 20.8% (23). In a recent study by Simbolo *et al.* frequent copy gains were detected in *AKT1* (30.8%) and *PDGFRA* (platelet-derived growth factor receptor alpha: 28.8%) as well. Furthermore, gains were present at the *FOS* gene (transcription factor subunit; 36.5%), *KIT* (involved in cell proliferation, survival, migration and differentiation; 28.8%) and *KDR* (kinase insert domain receptor, involved in *VEGF* signalling; 28.8%) genes (15). Higher mutation rates in primary SI-NETs were associated with increased likelihood of recurrent liver metastases ($P<0.04$) (23). In a study by Francis *et al.* ($n=180$) including 48 cases from Banck *et al.*, heterozygous frame shift mutations of the cyclin-dependent kinase inhibitor 1B gene (*CDKN1B*) in 14 of 180 SI-NETs (8%; 95% CI 4.7–12.7%) were observed (24). *CDKN1B* is located on chromosome 12 and encodes the protein $p27^{Kip1}$, a cyclin-dependent kinase inhibitor (CKI), whose main function is to control the progression from G1 to S phase in the cell cycle. The reported mutations in this putative tumour suppressor gene in SI-NETs are loss-of-function truncating mutations throughout the gene; no hotspot has been identified.

A further study by Crona ($n=200$), confirmed the presence of *CDKN1B* mutations in 17 of 200 SI-NETs (8.5%) (95% CI 4.6–12.4%) (26). Mutational status did not appear to correlate with protein expression of $p27^{Kip1}$ and no immediate detectable impact on clinical phenotype and survival was found (26). Similarly, Shi *et al.* observed *CDKN1B* mutations in 10.9% of 55 SI-NETs and found no association between *CDKN1B* mutation, $p27^{kip1}$ expression and survival (27). Only a trend towards shorter survival of patients with tumours exhibiting low expression of $p27^{kip1}$ (multivariate HR, 2.04; 95% CI 1.06–3.93; $P=0.03$) was observed. Other studies found *CDKN1B* mutations in 4.5–9.6% of SI-NETs (11, 15, 28, 29). Furthermore, Yao *et al.*, using whole-exome and targeted panel sequencing on 89 SI-NETs from the RADIANT trials, found recurring mutations in *BCOR* (BCL-6-interacting corepressor) in 5.6% (11). *BCOR* has interactions with histone deacetylases which are involved in the regulation of gene expression through DNA methylation (11). Another recent study, by Simbolo *et al.*, performed targeted sequencing on 52 primary SI-NETs of which 34.6% showed somatic mutations (15). *APC* (adenomatous polyposis coli; Wnt signalling pathway regulator) and *CDKN2C*

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(CKI 2C; cell growth regulator which controls cell cycle G1 progression) were found to be recurrently mutated in 7.7%. In addition, mutations were found in known oncogenes such as *BRAF* (involved in *MAPK/ERK* signalling pathway), *KRAS* (involved in *RAS/MAPK* signalling pathway), *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; involved in the *P13K/AKT/mTOR* pathway) and *TP53* (tumour suppressor gene; regulator of cell proliferation and apoptosis) in 3.8% (15). Previously, Bottarelli *et al.* ($n=30$) described *APC* gene alterations in 23% of ileal NETs (30). A copy gain of the *SRC* gene (proto-oncogene; involved in cell signalling), which was present in 25% of SI-NETs, was associated with poorer prognosis ($P=0.047$) (15). The *SRC* gene (cell signalling, cell cycle control and cell adhesion) was also the most commonly amplified oncogene (23%) in the study by Banck *et al.* (23). Copy gains of the *SRC* gene could potentially be a novel prognostic biomarker, especially as whole-genome sequencing becomes more widely adopted into clinical practice of SI-NETs.

Molecular alterations in primary tumours vs metastases

Molecular differences between primary tumours vs metastases can provide insight in the process of tumour progression. Cunningham *et al.* observed increased gains of chromosome 7 in metastases (30 mesenterial and 4 hepatic) vs primary SI-NETs (16 vs 0%) (19). Correspondingly, Hashemi *et al.* reported frequent gain of 7q22.3-qter in metastases (12 regional and 7 distant; $P=0.016$) compared to primary tumours (14). Loss of 16q12.2qter was more common in distant metastases vs primary tumours ($P=0.003$) (14). Karpathakis found LOH of chromosome 18 in 79% in liver metastases (31). In the same study, amplification of chromosome 20 was found in 42%, deletion of chromosome 19 in 34% and gain of chromosome 17q in 21% of liver metastases (31). Furthermore, at mRNA level, analysis of differentially expressed genes between liver metastases and primary tumours identified significant enrichment of multiple cancer-related pathways overexpressed in liver metastasis, for example *P13K* signalling events, *ERBB1* downstream signalling, *PDGFRB* signalling and the *mTOR* pathway (adjusted $P<0.001$) (31). Keck *et al.* demonstrated by RNA sequencing that liver metastases show underexpression of *PMP22* (peripheral myelin protein 22; integral membrane protein involved in demyelinating disease and apoptosis) compared to the corresponding primary tumour (<0.001) (32). Fotouhi *et al.* ($n=33$) found different expression levels of *CXCL14* (chemokine CXC motif ligand 14; involved in cytokine activity and angiogenesis) mRNA in metastases compared to primary tumours ($P=0.0016$), which correlated with methylation status of the respective genes (33). Furthermore, increased expression was found for mRNA encoding beta-catenin (involved in Wnt signalling pathway) in metastases compared with primary tumours ($P=0.041$); for mRNA encoding P16 (regulates entry into S phase) in distant metastasis compared to primary tumours and regional metastases ($P=0.015$) and for mRNA encoding RASSF1A (involved in cell cycle regulation, apoptosis and migration), in regional metastases compared to primary tumours and distant metastases ($P=0.008$). Low mRNA expression of RASSF1A and P16 were each associated with short survival ($P=0.045$ and $P=0.011$, respectively) (33). Using gene expression arrays, Edfeldt *et al.* were able to identify differentially expressed mRNA in SI-NET metastases compared to primary tumours which resulted in the identification of three different gene expression clusters. However, these clusters did not correlate with tumour progression (34). To conclude, dissemination of SI-NETs is associated with genomic events; yet the way in which these events contribute to tumour progression remains unclear.

Prognostic stratification based on LOH of chromosome 18, *CDKN1B* mutations, CpG island methylator phenotype and copy number variations

Karpathakis *et al.* identified different prognostic subgroups using hierarchical clustering. In a sophisticated large-scale integrated genomic analysis, including DNA methylation, gene expression and copy number variance (CNV) of 97 SI-NETs from a cohort of 85 patients they identified three molecular subtypes of SI-NETs using an integrated genome analysis (29). The largest subgroup (55%) was defined by chromosome 18 LOH and is associated with the presence of *CDKN1B* mutations, and CpG island methylator phenotype (CIMP) negativity. The CpG island methylator phenotype refers to the DNA hypermethylation of promoter-associated CpG islands of tumour suppressor and DNA repair genes, which leads to transcriptional silencing of these genes. These patients had the most favourable PFS (not reached at 10-year follow-up) after resection and a median age of 67 years at diagnosis. A second subgroup (18%) was characterised by the absence of arm-level CNVs (copy number variations that span the chromosomal arm) and a high degree of CIMP positivity. This group had an intermediate PFS (56 months) and a younger median age at diagnosis (60 years). The third subgroup consisted of

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26% of SI-NETs and was characterised by multiple CNVs; these patients had a significantly poorer PFS (21 months) and were youngest at onset (54 years), suggesting a more aggressive clinical phenotype. In accordance with Karpathakis *et al.*, Yao *et al.* (n=89), identified similar prognostic groups regarding LOH of chromosome 18 and alterations in chromosome 4, 5 and 20 (11). However, Simbolo *et al.* classified their cohort (n=52) into the three molecular groups of Karpathakis *et al.* and did not observe any statistically significant correlation with prognosis (P=0.73) (15). These results of Simbolo *et al.* could be due to the relatively small cohort in comparison to the cohort of Karpathakis *et al.* and do not necessarily weaken the findings of Karpathakis and Yao *et al.* Based on the findings of Karpathakis *et al.* and considering the relatively low frequency of somatic mutations in SI-NETs, it seems unlikely that mutations of the *CDKN1B* gene or LOH of chromosome 18 alone are driving the SI-NET tumorigenesis and suggests a greater role for epigenetic dysregulation (11, 15, 20, 25, 29, 35).

Germline mutations in SI-NETs

A germline mutation is defined as a mutation which occurs in reproductive cells and therefore is incorporated in every cell of the offspring. A study by Dumanski *et al.* (n=239) sequenced germline DNA from 24 patients from 15 families with a history of SI-NETs and from 215 sporadic SI-NET patients (36). A mono-allelic mutation causing an amino-acid substitution p. (Gly396Asp) in *MUTYH* was found to be significantly enriched in both patients affected with familial SI-NETs and in sporadic SI-NETs, compared to controls (minor allele frequencies 0.013 and 0.03, respectively) with an odds ratio of 5.09 (95% CI 1.56–14.74; P=0.0038). *MUTYH* encodes a DNA glycosylase, involved in repair of oxidative DNA damage in order to prevent mutation accumulation leading to tumorigenesis. Biallelic germline *MUTYH* mutations lead to multiple colorectal adenomas and carcinomas, referred to as MUTYH-associated polyposis (MAP), a recessive hereditary colorectal polyposis syndrome. Interestingly, *MUTYH* germline mutations were also found in pancreatic NETs (10, 37). By defective DNA repair, carriers with *MUTYH* mutations thus seem to have a predisposition to develop NETs of the pancreas or small intestine.

Epigenetics in SI-NETs

Epigenetic modification can be defined as a change in gene expression without alterations to the gene's DNA sequence itself (38). Since SI-NETs appear to have relatively few somatic mutations, epigenetic dysregulation could play an important role in the tumorigenesis of SI-NETs and may have important clinical implications (23, 24, 35). Epigenetic changes include DNA methylation, histone modifications and the actions of miRNA. Hypermethylation and hypomethylation and histone modifications modify gene expression, whereas miRNAs, small single-stranded RNA molecules, regulate gene expression post-transcriptionally.

These processes can be pharmacologically modified by targeting enzymes involved in DNA methylation and histone modifications, and by miRNA inhibitors, thereby representing an appealing target for therapy (35, 39).

In comparison with genetic mutations, epigenetic alterations are significantly more common and recurrent in SI-NETs. Our search yielded studies ranging from 8 to 97 patients that showed epigenetic alterations in SI-NETs for example DNA methylation changes in 65–82% of SI-NETs and multiple miRNA deregulations (29, 40, 41, 42). Several studies reported differences in methylation and miRNA patterns between primary tumours and metastases, suggesting a possible role in tumour development or progression. A recurrent event is the epigenetic silencing of *RASSFIA* (RAS-association domain family 1, isoform A gene; tumour suppressor gene inducing cell cycle arrest) expression by hypermethylation of its promotor. This event was observed by Choi *et al.*, Zhang *et al.* and Fotouhi *et al.* and was more prominent in metastases than in primary tumours (33, 43, 44). In addition, increased hypermethylation of the *CTNNB1* promoter was observed in liver metastasis compared to the corresponding primary tumours (44). Promotor gene methylation was also found in a study by Bollard *et al.* (n=38) in 96% of ileal NETs and their metastases. The expression of the axon guidance molecule SEMA3F (semaphorin 3F) was lost due to hypermethylation (45). SEMA3F expression is a negative regulator of *MAPK* and *mTOR* signalling pathways.

The first genome-wide DNA methylation analysis of SI-NETs performed by Verdugo *et al.* in 10 SI-NETs and ten matched mesenteric lymph node metastasis observed a high level of methylation in another gene set located at chromosome 18q21-qter (46). In these patients, high methylation index correlated with more malignant behaviour.

Karpathakis *et al.* found hypermethylation of the promoter region of the gastric inhibitory polypeptide receptor (*GIPR*; inhibits gastric secretion and gastrin release and stimulates insulin release) gene body in 74% of primary SI-NETs. Of note, in this study DNA

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methylation in SI-NETs was compared to the methylation status of normal intestinal mucosa which normally expresses GIPR, whereas the methylation status of enterochromaffin cells in the small intestine is unknown. Progressive hypermethylation of this gene was seen in liver metastases compared to primary tumours (29, 31).

MicroRNAs in primary tumours vs. metastases

Two miRNA profiling studies ($n=8$, $n=24$, respectively) comparing primary SI-NETs to its respective metastases found multiple miRNAs to be deregulated during tumour progression (40, 47). A downregulation of miRNA-133a and upregulation of miR-183 was consistently found in metastases vs primaries. A study by Miller *et al.* ($n=28$) confirmed downregulation of miRNA133a and found differential expression of several other miRNAs in SI-NETs and their metastases (48).

Discussion

SI-NETs are rare tumours with a relatively indolent course. Unfortunately, treatment options are limited with minimal survival benefit. Therapies targeting somatostatin receptors, expressed by the majority of SI-NETs, are only able to stagnate disease progression temporarily. In an attempt to identify prognostic factors and new effective targets for precision medicine, the genomic landscape of SI-NETs has been under increasing investigation in recent years. LOH at chromosome 18 remains the most frequent genomic aberration (44–100%) found in SI-NETs (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22).

The tumour suppressor gene, *CDKN1B*, is mutated in approximately 8% SI-NET patients (11, 15, 26, 27, 28, 29). Interestingly, *CDKN1B* is regulated by menin, the protein that is defect in the majority of patients with the inheritable MEN 1 syndrome (75–80%). Moreover, in MEN1 patients without mutations in the gene encoding menin (20–25%), *CDKN1B* was shown to be inactivated in some individuals (3.6%) (49). Thus, several SI-NETs and MEN1-associated endocrine tumours may share a common oncogenic pathway. Genetic alterations in the *P13K/AKT/mTOR* were found in primary SI-NETs and liver metastasis, providing a rationale for the use of mTOR inhibitors (15, 23, 31, 45). However, a correlation between efficacy and *mTOR* mutational status prior to commencement of therapy with mTOR inhibitors has not yet been established. Daskalakis *et al.* ($n=27$) recently tested the *ex vivo* activity of several targeted kinase inhibitors and found great variability in *ex vivo* sensitivity for most drugs, emphasising the need for predictive biomarkers which could support clinical decision making (50).

Furthermore, mutations in *APC*, *CDKN2C* (both 7.7%) and *BRAF*, *KRAS*, *PIK3CA* and *TP53* (each 3.8%) were recently identified in SI-NETs (15).

An association of (epi)genetic aberrations with prognosis was found in 16 of the 35 original studies reviewed. Karpathakis *et al.* ($n=97$) and Yao *et al.* ($n=89$) identified molecular subtypes of SI-NETs with significant difference in PFS (11, 31). However, validation of these subgroups in an independent and larger cohort is required before translation into clinical practice is possible. A gain of chromosome 14 and 20pter-p11.21 was associated with shorter survival in two studies ($P<0.001$, $P<0.013$ respectively) (13, 14). *SRC* copy number gains were associated with poorer prognosis ($P=0.047$) (11). Epigenetic alterations such as specific promotor methylation and global methylation and their effect on prognosis are yet to be determined (33, 41, 43, 44, 45, 46).

At present, predictive or prognostic biomarkers, which can be adopted into clinical practice, have not yet been established. Inactivated tumour suppressor genes, which are found in SI-NETs, are generally unsuitable as targets since restoring the function of tumour suppressor genes is difficult to accomplish. Mutations in oncogenes, which should be easier to target, have only recently been described in small numbers in SI-NETs and thus far no clinical studies have been undertaken to target these mutations in SI-NETs. Of note, Alvarez *et al.* identified the HDAC class inhibitor Etinostat as potent inhibitor of master regulatory activity for 42% of metastatic gastroenteropancreatic NETs, leading to the initiation of a clinical trial (NTC03211988) (9).

The low mutational burden found in SI-NETS may render these tumours less eligible for immunotherapy using immune checkpoint inhibitors because tumour mutational burden is an important determinant of clinical benefit to immune checkpoint blockade in most tumours. Additionally, the recently characterised tumour microenvironment in NETs, for example low expression for PD1 and PDL1 in SI-NETs, combined with a modest T-cell infiltrate, further tempers expectations regarding a response to the currently used PD1 and PDL-1 inhibitors, although this remains to be investigated (51).

More promising targets in SI-NETs may constitute the DNA methylation machinery. In comparison with genetic mutations, epigenetic alterations are significantly more common in SI-NETs. Specific genes such as *RASSF1A*, *SEMA3F* and *CTNNB1* are hypermethylated in SI-NETs silencing their transcription (3, 43, 44, 45, 46).

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RASSF1A hypermethylation is also observed in pancreatic NETs, lung NETs and thymic NETs, whilst it is not found in appendiceal NETs (52). During the last decades, an increasing number of drugs targeting DNA methylation and histone methylation have been developed and successfully tested pre-clinically which are currently in evaluation in phase I-III clinical trials (53). Additionally, the more specific upregulation of miRNAs in SI-NETS as described above may provide actionable targets since multiple strategies for miRNA-based therapies are under investigation (54).

In this era of accumulating studies regarding the molecular background of SI-NETs, we felt there was an unmet need to provide the clinician with an overview of (epi)genetic alterations and explain their relevance in terms of prognosis and possible novel therapeutic options. Despite our efforts to perform an extensive and broad search, studies may have been missed due to its non-systemic character. Limitations of studies used in this review include relatively small and heterogeneous cohorts, different genomic analysis techniques and paucity of relation of (epi)genetic aberrations to clinical outcomes.

The rarity of SI-NETs has hampered conducting sizeable clinical trials involving large-scale integrated genomic analysis. In the coming years, hopefully international collaborations will enable larger studies to be performed which correlate (epi)genetic alterations to clinical outcomes and aim to identify targetable (epi)genetic alterations. Larger studies combined with evolving molecular technologies might lead to a more effective treatment strategy in which patients with specific molecular tumour profiles will be selected for targeted pharmaceutical interventions.

Conclusion

SI-NETs have a low mutational burden, whereas epigenetic alterations are more prevalent. Mutations as described in pancreatic NETs are generally not observed in SI-NETs. Several studies identified (epi)genetic subtypes and molecular profiles of SI-NETs with significant difference in progression-free survival (PFS) and overall survival (OS). More research should be conducted to identify prognostic and predictive biomarkers that can be adopted in clinical decision making.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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