

# Mutational Analysis of *p27* (CDKN1B) and *p18* (CDKN2C) in Sporadic Pancreatic Endocrine Tumors Argues against Tumor-Suppressor Function<sup>1</sup>

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## Abstract

**Pancreatic endocrine tumors (PETs) arise sporadically or are associated with multiple endocrine neoplasia type 1 (MEN1) syndrome or von Hippel-Lindau syndrome. About 90% of patients with familial MEN1 display detectable *MEN1* gene (menin) mutations. The cyclin-dependent kinase inhibitor *p27* (CDKN1B) is a downstream target of menin and has been recently shown to be responsible for the multiple endocrine neoplasia–like syndrome in rats, where affected animals develop multiple tumors and hyperplasia in endocrine tissues, including the pancreatic islets of Langerhans. A germline nonsense truncation mutation of *p27* has been recently described in a suspected MEN1 family without MEN1 mutation, raising the possibility that *p27* mutation could be responsible for MEN1 phenotype. Somatic MEN1 mutations occur at low frequency in sporadic PETs; here, we subjected *p27* to mutational analysis in 27 sporadic PETs. As an additional menin target, analysis of the *p18* (CDKN2C) gene was included. In the *p27* gene, one common polymorphism (V109G) and one novel polymorphism (g/a) in the noncoding part of exon 2 were identified. Three known polymorphisms were found in the *p18* gene. These data suggest that *p27* and *p18* are unlikely to present classic tumor-suppressor genes in sporadic PETs.**

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**Keywords:** cyclin-dependent kinase inhibitor, p27Kip1, p18INK4C, sporadic pancreatic endocrine tumors, tumor-suppressor gene.

## Introduction

Pancreatic endocrine tumors (PETs) constitute benign and malignant lesions that are classified according to symptoms of hormonal excess or are considered nonfunctioning. PETs arise sporadically from or in association with multiple endocrine neoplasia type 1 (MEN1) syndrome and von Hippel-Lindau syndrome. The prevalence of detected germline mutations of the *MEN1* gene on chromosome 11q13 in MEN1 families is about 90%, and affected individuals classically suffer from tumors of the pituitary gland, parathyroid glands, and endocrine pancreas [1–5]. The *MEN1* gene is less frequently mutated in sporadic PETs [6–8].

The gene responsible for the multiple endocrine neoplasia–like syndrome in rats has been recently identified as the cyclin-dependent kinase inhibitor *p27*. The affected animals developed endocrine pancreatic hyperplasia, parathyroid adenomas, paragangliomas, bilateral pheochromocytomas, and multifocal thyroid C-cell hyperplasia [9]. A germline nonsense truncation mutation in *p27* was detected in several members of a family with a variant of the MEN1 syndrome, with tumors of the parathyroid and pituitary glands, and without identifiable MEN1 mutation. Based on these findings, it was suggested that germline mutations in *p27* can predispose to the development of multiple endocrine tumors in both rats and humans [9,10]. Experiments in mice also support a tumorigenic role of *p27*, as well as of *p18*. Knockout of the *p18* or the *p27* gene in mice caused widespread hyperplasia and organomegaly, as well as tumor formation [11]. Double *p18/p27* knockout mice developed multiple tumors of endocrine organs, including pancreatic islet hyperplasia [11]. Double *p27/MEN1* knockout mouse models have demonstrated that combined gene loss did not increase pancreatic islet tumor development, whereas double *p18/MEN1* knockout mice developed increased rate of tumors [12]. Menin, the product of the *MEN1* gene, has been shown to positively regulate the transcription of the *p27* and *p18* genes in mouse pancreatic islets [13,14]. We examined the *p27* and *p18* gene mutational status in sporadic human PETs.

## Materials and Methods

### Patients and Tumor Samples

Twenty-seven patients were recruited and tissue specimens were collected during clinical routine at the Uppsala University Hospital, with informed consent and approval of the ethics committee. Twenty-seven sporadic PETs were investigated, including 10 benign insulinomas, 3 malignant insulinomas, 10

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malignant nonfunctioning tumors, 3 malignant glucagonomas, and 3 malignant gastrinomas. All investigated tumors were primary tumors, except for tumor 5516, where lymph node metastasis was observed (Table 1).

#### Polymerase Chain Reaction (PCR) and DNA Sequencing Analysis

Tumor DNA was extracted from cryosections by standard procedures after microdissection to avoid gross contamination by nontumor cells. Tumor cells were identified by immunohistologic staining using a synaptophysin rabbit polyclonal antibody (A0010; DAKO A/S, Glostrup, Denmark). Coding parts of the *p27* and *p18* genes were investigated using previously published primers [15]. PCR reactions were conducted on tumor DNA and blood DNA corresponding to tumor 3219 (new *p27* gene polymorphism; see below), as described previously [16], and dimethylsulfoxide was used when necessary. Amplified PCR fragments were subjected to sequencing on a 3130XL Genetic Analyzer using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). Three known polymorphisms in the *CDKN2C/p18* gene were detected (Table 1): contig positions 21411375 ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=3176471](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=3176471)), 21411696 ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=1043141](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1043141)), and 21412012 ([http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=12855](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=12855)) in contig

NT\_032977. The common V109G polymorphism in the *p27* (*CDKN1B*) gene was detected at the expected frequency (5630073 in contig NT\_009714; [http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=2066827](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2066827)), as well as an unknown polymorphism in a noncoding part of exon 2 [5630867 (g/a) in contig NT\_009714].

#### Results and Discussion

Direct sequencing of the *p27* and *p18* genes was conducted on 27 sporadic PETs. A previously unpublished polymorphism was detected with a g/a nucleotide change at 13 bp after the stop codon in the *p27* gene (5630867 in contig NT\_009714). In addition, one (5630073 in contig NT\_009714) and three (21412012, 21411696, and 21411375 in contig NT\_032977) known polymorphisms were found in *p27* and *p18*, respectively (Table 1; see also Materials and Methods section). All other sequences corresponded to GenBank. These findings do not support a role for *p27* and *p18* as classic tumor-suppressor genes in sporadic PETs.

In addition, a recent report found no evidence of the involvement of *p27* in 16 index cases of the MEN1 variant with tumors of both parathyroid and pituitary glands and no identified MEN1 mutation [17].

Whether *p27* presents a crucial target in other endocrine malignancies remains to be determined.

**Table 1.** Characterization of PETs Analyzed in This Study.

Tumor Number	Tumor Syndrome	Signs of Malignancy	Follow-up (Years)	<i>p18</i> Polymorphism			<i>p27</i> Polymorphism	
				21412012	21411696	21411375	5630073	5630867
3945	Insulinoma	None	FD (14)	ct	ct	aa	gt	gg
4642	Insulinoma	None	FD (13)	ct	ct	aa	tt	gg
4741	Insulinoma	None	FD (13)	cc	cc	aa	tt	gg
5735	Insulinoma	None	FD (12)	cc	cc	aa	tt	gg
6832	Insulinoma	None	FD (8)	ct	ct	aa	gt	gg
7436	Insulinoma	None	FD (6)	cc	cc	aa	tt	gg
7602	Insulinoma	None	FD (6)	cc	cc	aa	tt	gg
7701	Insulinoma	None	DO (4)	ct	ct	aa	gg	gg
8113	Insulinoma	None	FD (4)	ct	ct	aa	gt	gg
8341	Insulinoma	None	FD (3)	cc	cc	aa	gt	gg
117	Insulinoma	Lymph node metastases	AWD (22)	cc	cc	aa	tt	gg
8495	Insulinoma	Lymph node metastases*	AWD (3)	cc	cc	at	gt	gg
8734	Insulinoma	Infiltrative growth	NR (2)	ct	ct	aa	gt	gg
7495	Gastrinoma	Liver metastases	AWD (6)	cc	cc	aa	tt	gg
2576	Glucagonoma	Liver metastases	AWD (17)	cc	cc	aa	tt	gg
5516	Glucagonoma	Lymph node metastases†	DRD (7)	cc	cc	aa	tt	gg
8640	Glucagonoma	Infiltrative growth	NR (3)	cc	cc	aa	gt	gg
2663	Nonfunctioning tumor	Lymph node metastases‡	DRD (10)	cc	cc	aa	tt	gg
3219	Nonfunctioning tumor	Liver metastases	DRD (7)	cc	cc	aa	tt	ag
5287	Nonfunctioning tumor	Lymph node metastases§	AWD (12)	cc	cc	aa	gt	gg
5620	Nonfunctioning tumor	Liver metastases	DRD (7)	cc	cc	aa	gt	gg
6114	Nonfunctioning tumor	Infiltrative growth¶	AWD (11)	cc	cc	aa	gt	gg
7482	Nonfunctioning tumor	Liver metastases	DRD (1)	cc	cc	aa	gg	gg
7536	Nonfunctioning tumor	Lymph node metastases	NR (6)	cc	cc	aa	gt	gg
8227	Nonfunctioning tumor	Liver metastases	AWD (4)	ct	ct	aa	tt	gg
8357	Nonfunctioning tumor	Liver metastases	AWD (3)	cc	cc	aa	gg	gg
8970	Nonfunctioning tumor	Liver metastases	NR (2)	cc	cc	aa	tt	gg

FD, free of disease; DO, death due to other causes; AWD, alive with disease; NR, no signs of recurrence; DRD, disease-related death.

\*Liver metastases detected after 2 years.

†Liver metastases detected after 6 years.

‡Liver metastases detected after 7 years.

§Liver metastases detected after 8 years.

¶Liver metastases detected after 7 years.

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