Whole-exome characterization of pancreatic neuroendocrine tumor cell lines BON-1 and QGP-1

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Abstract

The human BON-1 and QGP-1 cell lines are two frequently used models in pancreatic neuroendocrine tumor (PNET) research. Data on the whole-exome genetic constitution of these cell lines is largely lacking. This study presents, to our knowledge, the first whole-exome profile of the BON-1 and QGP-1 cell lines. Cell line identity was confirmed by short tandem repeat profiling. Using GTG-banding and a CytoSNP-12v2 Beadchip array, cell line ploidy and chromosomal alterations were determined in BON-1 and QGP-1. The exomes of both cell lines were sequenced on Illumina's HiSeq next-generation sequencing (NGS) platform. Single-nucleotide variants (SNVs) and insertions and deletions (indels) were detected using the Genome Analysis ToolKit. SNVs were validated by Sanger sequencing. Ploidy of BON-1 and QGP-1 was 3 and 4 respectively, with long stretches of loss of heterozygosity across multiple chromosomes, which is associated with aggressive tumor behavior. In BON-1, 57 frameshift indels and 1725 possible protein-altering SNVs were identified in the NGS data. In the QGP-1 cell line, 56 frameshift indels and 1095 SNVs were identified. ATRX, a PNET-associated gene, was mutated in both cell lines, while mutation of TSC2 was detected in BON-1. A mutation in NRAS was detected in BON-1, while KRAS was mutated in QGP-1, implicating aberrations in the RAS pathway in both cell lines. Homozygous mutations in TP53 with possible loss of function were identified in both cell lines. Various MUC genes, implicated in cell signaling, lubrication and chemical barriers, which are frequently expressed in PNET tissue samples, showed homozygous protein-altering SNVs in the BON-1 and QGP-1 cell lines.

Key Words
- BON-1
- QGP-1
- PNET
- whole-exome sequencing
- SNP array

Introduction

Pancreatic neuroendocrine tumors (PNETs) are malignant tumors probably arising from islet cells of the pancreas (Schimmack et al. 2011). PNETs can secrete a variety of hormones including somatostatin, insulin, glucagon, serotonin, and pancreatic polypeptide. The majority of PNETs, however, are nonsecreting (Yao et al. 2008). In the USA, the age-adjusted annual incidence of PNET is 0.32/100 000 and the standardized prevalence is
5/100,000, according to the Surveillance Epidemiology and End Results (SEER) data (Yao et al. 2008). In PNET, surgical resection alone is often curative in early-stage disease, but 40% to 50% of PNET patients have advanced disease at the time of initial diagnosis or present with synchronous tumors (Yao et al. 2008). Patients with advanced disease may suffer from complications of uncontrolled hormone secretion and usually succumb to these complications or tumor progression (Kulke et al. 2011).

PNETs are associated with a number of inherited syndromes that are characterized by mutations in well-studied proto-oncogenes or tumor-suppressor genes. These include multiple endocrine neoplasia types 1 and 2 (MEN1 and MEN2), von Hippel–Lindau disease (VHL), and tuberous sclerosis (TSC). However, the majority of PNETs occurs as sporadic tumors (Leotlela et al. 2008). These include multiple endocrine neoplasia types 1 and 2 syndromes that are characterized by mutations in well-studied proto-oncogenes or tumor-suppressor genes.

To allow in vitro study of PNET, the BON-1, QGP-1, and CM human cell lines have been developed. Kaku et al. (1980) established the QGP-1 cell line from primary PNET tumor tissue obtained from a 61-year-old male. The BON-1 cell line was established by Townsend and colleagues in 1986 from a peripancreatic lymph node metastasis of a 28-year-old male with PNET (Townsend et al. 1993). In 1987, the CM cell line was established from the peritoneal ascites of a patient with pancreatic islet cell tumors. The BON-1 and CM cell lines were obtained from Professor Dr Townsend (University of Texas Medical Branch, Galveston, TX, USA). Both cell lines were confirmed as Mycoplasma-free using MycoAlert (Lonza, Verviers, Belgium). The BON-1 cell line was cultured in 1:1 mixture of DMEM and Ham’s F-12K (Kaighn’s) medium, supplemented with 10% (v/v) FCS, penicillin (1 × 10^5 units/l), fungizone (0.5 mg/l), and L-glutamine (2 mmol/l). The QGP-1 cell line was cultured in RPMI-1640 medium, supplemented with 10% (v/v) FCS and penicillin–streptomycin (1 × 10^5 units/l). All cell lines were cultured in a humidified incubator at 5% CO_2 and 37 °C. The media and supplements were obtained from Life Technologies (Invitrogen). DNA from BON-1 (passage number 9) and from QGP-1 (passage number 14) was isolated using a QIAamp DNA Mini Kit (Qiagen), following the manufacturer’s instructions. The concentration of the isolated DNA was quantified using a Qubit 2.0 fluorometer with a dsDNA Broad Range Assay (Thermo Scientific, Waltham, MA, USA). A high-molecular-weight band was visible on DNA gel electrophoresis, excluding DNA degradation. Short tandem repeat profiling using a Powerplex Kit (Promega) of BON-1 and QGP-1 gave results consistent with those obtained by the original group (Silva et al. 2011) and the JRCB database, respectively, thus confirming the cell lines’ identity.

Materials and methods

Cell culture and DNA isolation

The QGP-1 cell line was purchased from the Japanese Collection of Research Bioresources Cell Bank (JRCB, Osaka, Japan). The BON-1 cell line was a kind gift from Dr Townsend (University of Texas Medical Branch, Galveston, TX, USA). Both cell lines were confirmed as Mycoplasma-free using MycoAlert (Lonza, Verviers, Belgium). The BON-1 cell line was cultured in 1:1 mixture of DMEM and Ham’s F-12K (Kaighn’s) medium, supplemented with 10% (v/v) FCS, penicillin (1 × 10^5 units/l), fungizone (0.5 mg/l), and L-glutamine (2 mmol/l). The QGP-1 cell line was cultured in RPMI-1640 medium, supplemented with 10% (v/v) FCS and penicillin–streptomycin (1 × 10^5 units/l). All cell lines were cultured in a humidified incubator at 5% CO_2 and 37 °C. The media and supplements were obtained from Life Technologies (Invitrogen). DNA from BON-1 (passage number 9) and from QGP-1 (passage number 14) was isolated using a QIAamp DNA Mini Kit (Qiagen), following the manufacturer’s instructions. The concentration of the isolated DNA was quantified using a Qubit 2.0 fluorometer with a dsDNA Broad Range Assay (Thermo Scientific, Waltham, MA, USA). A high-molecular-weight band was visible on DNA gel electrophoresis, excluding DNA degradation. Short tandem repeat profiling using a Powerplex Kit (Promega) of BON-1 and QGP-1 gave results consistent with those obtained by the original group (Silva et al. 2011) and the JRCB database, respectively, thus confirming the cell lines’ identity.

SNP array

BON-1 and QGP-1 DNA was hybridized on a CytoSNP-12v2 Beadchip array (Illumina, San Diego, CA, USA), after whole-genome amplification with an Infinium HD assay (Illumina) using 200 ng input DNA, according to the manufacturer’s protocol. The array was scanned using an Illumina iScan (Illumina). The resulting scan data were imported, mapped, and clustered using Illumina GenomeStudio Software v2011.1 equipped with the genotyping module (version 1.9.4) (Illumina), before being exported for further analysis using allele-specific copy number analysis of tumors (ASCAT) (Van Loo et al. 2010) with default parameters. ASCAT takes both aneuploidy of the tumor cells and nonaberrant cell infiltration into account when analyzing SNP array data. This allows identification of copy number variants (CNVs), copy-number neutral events, and loss of heterozygosity (LOH) in nondiploid tumor samples, which cannot be picked up by noncorrected SNP array analysis algorithms.

Preparation of metaphase spreads and GTG-banding

Growth of BON-1 and QGP-1 cells in their respective media was synchronized by adding 100 nM 5-fluor-
2-deoxyuridine and 4 μM uridine overnight followed by 10 μM thymidine after 15 h. To prepare metaphase spreads, the synchronized cultures were arrested in mitosis by adding demecolcine (1 μg/ml) for 10 min at 37 °C. By incubating the cells in 75 μM KCl at 37 °C for 20 min, a hypotonic shock was applied after which the cells were fixed in methanol/acetic acid (3:1, v/v) and washed twice. Metaphase spreads on uncoated glass slides were air-dried and thermally aged for 1 h at a temperature of 80–100 °C. Giemsa-banding (GTG-banding) was executed using trypsin and eosin–polychromic-methylene blue solution (Leishman solution) according to the standard protocol (Seabright 1971). Chromosomal abnormalities identified by GTG-banding in 20 metaphases were described according to the guidelines of the International System for Human Cytogenetic Nomenclature (Shaffer et al. 2009).

Whole-exome sequencing

Two micrograms of isolated BON-1 and QGP-1 DNA were fragmented using a focused-ultrasonicator (Covaris M220, Covaris, Woburn, MA, USA) running the following program: duty factor of 20%, peak power of 50 with 200 cycles/burst during 120 s. Next, all samples were prepared for exome sequencing using a TruSeq DNA Sample Preparation Kit (Illumina) following the low-throughput, gel-free protocol according to the manufacturer’s instructions. DNA exonic sequences were enriched using the Truseq Exome Enrichment Kit (Illumina) according to the manufacturer’s instructions. The enriched samples were hybridized and amplified on the three lanes of a paired-end flow cell using Illumina’s cBot (Illumina). Next, all samples were exome sequenced using Illumina’s HiSeq 1500 (Illumina) platform.

Quality control and data analysis of whole-exome data

Raw sequencing reads were analyzed using an in-house developed Perl-based workflow. First, FastQC Software (version 1.0) was used to assess quality of the raw data. Adapters and low-quality bases were trimmed using Cutadapt (version 1.2.1) and an in-house developed quality trimmer (Helsmoortel et al. 2014) respectively. Paired-end reads were then aligned to the human reference genome (hg19, NCBI Build 37) using a Burrows–Wheeler Aligner (BWA mem, version 0.7.4) (Li & Durbin 2009). Picard (version 1.88) was used to mark and remove duplicates. Using the Genome Analysis Toolkit (GATK version 2.8.1) (McKenna et al. 2010), insertions and deletions (indels) were realigned and recalibrated. Single-nucleotide variants (SNVs) and indels were detected using GATK, annotated with ANNOVAR (Wang et al. 2010), and filtered using VariantDB (Vandeweyer et al. 2014) according to different criteria (see Results section for details). Integrative Genomics Viewer (IGV, version 2.2.5) was used to visualize the reads (Thorvaldsdottir et al. 2012). Data were visualized using Circos Software (version 0.66) (Krzywinski et al. 2009) and made publicly available through European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB8223).

Results

Determination of ploidy and chromosomal aberrations

The genotyping results from the CytoSNP-12v2 Beadchip array were analyzed using the ASCAT algorithm, allowing correction for nondiploidy in samples from the two cell lines. ASCAT yielded an estimated average overall ploidy of 3 in BON-1 and 4 in QGP-1 (Fig. 1A and B). ASCAT analysis revealed multiple allelic duplications and deletions across the BON-1 and QGP-1 genome, resulting in frequent CNVs and regions with LOH (Fig. 1C and D). Long stretches of LOH were seen in chromosomes 1, 3, 4, 6, 9, 11, 17, 18, 22, and X in BON-1, while in QGP-1, LOH of nearly entire chromosomes was observed in chromosomes 7, 8, 9, 13, 17, 21, 22, and X. These stretches of LOH were also seen in our next-generation sequencing (NGS) results (see below). In the NGS data, LOH was defined as an average allelic fraction (AF) above 0.66 in BON-1 and above 0.75 in QGP-1, corresponding to the respective ploidy of 3 and 4 (Fig. 2).

In order to confirm the ploidy calculations of ASCAT, we karyotyped both cell lines. GTG-banding results for BON-1 and QGP-1 were consistent with the results from the CytoSNP-12v2 Beadchip array. The BON-1 GTG-banding showed a near-triploid karyotype with a modal chromosome number of 63. Extensive chromosomal rearrangements and multiple derivative chromosomes were observed (Supplementary Figure S1, see section on supplementary data given at the end of this article). This was in accordance with results published previously (Lopez et al. 2010). GTG-banding of the QGP-1 cell line revealed a near-tetraploid karyotype with a modal chromosome numbers of 90–92, including multiple derivative chromosomes, thus confirming the ASCAT profile (Supplementary Figure S2, see section on supplementary data given at the end of this article).

Detection and filtering of SNVs

Whole-exome resequencing of BON-1 generated 91.5 million reads, while sequencing of QGP-1 generated...
107.6 million reads. Of these, 57.9% of the BON-1 reads and 59.1% of the QGP-1 reads were mapped by BWA on targets enriched by the TruSeq Exome Enrichment Kit. The average base coverage for the target regions was 68! and 81! for BON-1 and QGP-1 respectively, with 92.9% of these nucleotides in BON-1 and 93.9% in QGP-1 covered at least ten times (Fig. 2). After indel realignment, a GATK Unified Genotyper was used for variant detection with adapted settings for polyploidic samples. Based upon the SNP array and GTG-banding results, a ploidy of 3 for BON-1 (A) and 4 for QGP-1 (B). Using the resulting tumor ploidy and aberrant cell fraction, an ASCAT profile was calculated (C and D, upper panels), containing the allele-specific copy number of all assayed loci. Copy number is shown on the y-axis vs the genomic location on the x-axis (light grey, allele with lowest copy number; dark grey, allele with highest copy number). For illustrative purposes only, both lines are slightly shifted (highest, down; lowest, up) such that they do not overlap. Only heterozygous SNPs are shown. Finally, for all aberrations that were found, an aberration reliability score was calculated (C and D, lower panels). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-14-0304.

Nucleotide substitution frequencies

In both BON-1 and QGP-1, the most frequent nucleotide substitutions are A→G or C→T transitions (Fig. 4). In BON-1, transitions made up 66.8% of the filtered SNVs, resulting in a twofold overrepresentation of transitions compared with transversions. In QGP-1, transitions
accounted for 64.7% of the filtered SNVs. The ratio of transitions to transversions was 1:1.8 for the filtered SNVs in QGP-1. Overall, the ratio of transitions to transversions was similar in BON-1 and QGP-1 and higher than the average transitions to transversion rate of 1:1.7 that is usually seen in human germline samples (Lynch 2010). A mutation spectrum with a predominant C to T/G to A transition pattern is seen in many adult cancers, including melanoma, breast, lung, colorectal, ovarian, and pancreatic adenocarcinomas (Greenman et al. 2007, Jones et al. 2008). However, in PNETs a more even distribution of transversions and transitions has been reported previously (Jiao et al. 2011).

Detection of small indels

On the indel-realigned and BWA-mapped reads, indels were identified using the GATK Unified Genotyper with the ploidy-settings adjusted to 3 for BON-1 and 4 for QGP-1. Quality filtering was executed with VariantDB using similar criteria to those for SNP filtering (Fig. 5). In BON-1, from the initial 2923 predicted insertions and 3194 predicted deletions, 20 insertions and 37 deletions were, after quality filtering, identified as RefSeq frameshift alterations, possibly leading to protein alterations. Of the initial 2749 predicted insertions and 2903 predicted deletions in QGP-1, 21 RefSeq frameshift insertions and 35 RefSeq frameshift deletions remained after quality filtering.

Prediction of effects on protein function

To further identify SNVs with effects on protein function and a possible role in oncogenesis, in silico prediction of the effects on protein function and structure was made using four different software packages: PolyPhen2, MutationTaster, SIFT, and PROVEAN (Kumar et al. 2009, Adzhubei et al. 2010, Choi et al. 2012, Schwarz et al. 2014). In BON-1, 61.5% of the filtered SNVs (1061 SNVs) had a predicted effect on protein function according to at least one of the four methods. In QGP-1, 67.2% of all filtered SNVs (736 SNVs) were predicted to be deleterious with respect to protein function by at least one of the four packages used. However, only 137 SNVs (7.9% of all filtered SNVs) were predicted to be deleterious by all four packages in BON-1, while 119 SNVs (10.8% of all filtered SNVs) in QGP-1 were predicted by all packages to have a deleterious effect on protein function. MutationTaster was used for prediction of effects on proteins of frameshift indels in BON-1 and QGP-1. In BON-1, this identified 34 indels (59.6% of identified indels) with a predicted
deleterious effect on protein function. In QGP-1, 23 indels (41.0% of identified indels) were predicted to be deleterious with respect to protein translation.

Comparison of BON-1 and QGP-1 exomes

When comparing the genomic positions of the identified SNVs and indels in BON-1 and QGP-1, 221 positions showed alterations in both QGP-1 and BON-1. Of these 221 genomic positions, QGP-1 and BON-1 showed the same nucleotide alterations in 216 positions (97.7%) while at five positions a different nucleotide substitution, insertion, or deletion were seen. Of all the alterations found at these 221 genomic positions, 24 alterations were predicted by all four protein prediction programs to be damaging in QGP-1, while in BON-1, 25 alterations were predicted to be protein-damaging. We aimed at elucidating underlying PNET pathways and mechanisms by comparing BON-1 and QGP-1, looking at alterations at different genomic positions but within the same gene in BON-1 and QGP-1. First a list of genes containing SNVs or indels with a predicted damaging effect on protein function was created for BON-1 and QGP-1 separately. This resulted in a gene list containing 134 genes for BON-1 and 119 genes for QGP-1. When the two lists were combined, 13 genes were identified that contained one or more mutations in both BON-1 and in QGP-1, including cancer-associated TP53 and CTBP2 (Takayama et al. 2014) (Supplementary Table 1, see section on supplementary data given at the end of this article).

Genetic validation of SNVs

Artifacts in next-generation whole-exome sequencing data could arise from different sources such as PCR-amplification-based enrichment, sequencing technology proper or even sample mix-up. As we used a stringent filtering strategy, the risk was minimized, although not fully excluded. Therefore, using Sanger sequencing, we validated a subset of 22 SNVs and four indels from genes that came up as mutated in BON-1 and QGP-1 (Table 2). All SNVs and indels were confirmed by this technique. Although this represents only a small portion of all identified SNVs and indels, it illustrates the low level of sequencing artifacts in our filtered datasets.

Discussion

Overview of genetic alterations

The presented extensive genetic analysis of the neuroendocrine tumor cell lines BON-1 and QGP-1 is, to our knowledge, the first study to combine karyotyping, SNP array, and NGS whole-exome data. In a previous study, BON-1 was characterized using GTG-banding, comparative genomic hybridization (CGH), and fluorescence in situ hybridization (FISH) (Lopez et al. 2010). These low-resolution methods only allowed the study of large structural chromosomal alterations and copy number variations for a limited set of selected genes. SNVs cannot be identified by the methods applied in these studies. No data on the genetic constitution of QGP-1 are present in literature. Our study not only confirms previous findings regarding BON-1, but also adds new genotypic data using high-resolution methods. SNP array and NGS allow identification of small structural variations such as gene copy number variations and nucleotide changes such as SNVs and indels throughout the genome. For both BON-1 and QGP-1, this is the first time, to our knowledge, that the genome has been studied in this detail, leading to insights into the genetic origins of the two cell lines and their neuroendocrine tumor characteristics.

Table 1 RefSeq variant type of filtered SNVs in BON-1 and QGP-1

<table>
<thead>
<tr>
<th>Variant-type filtered SNVs</th>
<th>BON-1 (percentage of total)</th>
<th>QGP-1 (percentage of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stopgain SNVs</td>
<td>33 (1.9)</td>
<td>26 (2.4)</td>
</tr>
<tr>
<td>Stoploss SNVs</td>
<td>3 (0.2)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Nonsynonymous SNVs</td>
<td>1689 (97.9)</td>
<td>1068 (97.5)</td>
</tr>
<tr>
<td>All filtered SNVs</td>
<td>1725 (100)</td>
<td>1095 (100)</td>
</tr>
</tbody>
</table>
Extensive LOH in BON-1 and QGP-1

In QGP-1 as well as BON-1, the SNP array and NGS results were indicative of an extensive LOH over multiple chromosomes. An increased frequency of LOH has been observed in 60 Caucasian human cancer cell lines derived from several organs and cell types including brain, CNS, colon, lung, white blood cells, melanocytes, ovary, prostate and kidney when compared with germline DNA of healthy subjects (Ruan et al. 2012). In addition, LOH in germline DNA is more frequent in patients with different tumor types in comparison with healthy controls (Assie et al. 2008). In a previous study, loss of CDKN2A and CDKN2B was identified in BON-1 through FISH (Lopez et al. 2010). The lack of reads in the NGS data at the respective CDKN2A and CDKN2B loci in BON-1 confirms these findings. CDKN2A and CDKN2B encode the tumor suppressors p16INK4A and p14ARF, respectively, and inactivation could lead to unrestrained cell growth (Rocco & Sidransky 2001). No CDKN2A and CDKN2B alterations were found in QGP-1. In neuroendocrine tumors, results from a recent study have indicated the importance of LOH of the PHLDA3 gene in tumor progression and poor prognosis in addition to existing evidence of the importance of LOH of the MEN1 gene for tumorigenesis (Corbo et al. 2010, Ohki et al. 2014). Results from previous studies indicated that MEN1 expression was very low in BON-1 and that a reduction in growth was found when BON-1 was transfected with MEN1 (Stalberg et al. 2004). In this study, SNP array and NGS analysis of BON-1 yielded results indicative of LOH of MEN1. In addition, LOH of PHLDA3 was observed in BON-1. In QGP-1, LOH was observed for MEN1, but not for PHLDA3. This difference indicates that the two cell lines might have other mechanisms causing their malignant transformation.

Figure 4
Mutation spectrum of single-nucleotide variants (SNVs) in BON-1 (left) and QGP-1 (right). Percentages of total number of predicted SNVs (light grey) and filtered SNVs (dark grey) in each of the six possible mutation classes. A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-14-0304.

Figure 5
Number of insertions and deletions (indels) per filter step. Quality and coding DNA: total reading depth ≥ 10, mapping quality ≥ 50, Fisher-scaled strand bias ≤ 20, allelic ratio ≥ 0.1, snpEff annotation = noncoding, Alternative allele-depth ≥ 2; novel mutations: only SNVs and indels with a MAF in NCBI dbSNP137 database > 0.05 and in the 1000 genomes 2012 > 0.05; protein-altering mutations: RefSeq frameshift indels. A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-14-0304.
TP53 of BON-1 and QGP-1 were Sanger sequenced. This limited sequencing experiment failed to identify any mutations (Bartz et al. 1996). However, in our NGS sequencing data, we found a homozygous (AF 99%) stopgain g.7574003A>G mutation (ENSP00000269305 p.R342X) in exon 10 of TP53 in BON-1 and a homozygous (AF 100%) g.7579394delG frameshift deletion (ENSP00000391127 p.P98Lfs*25) in exon 4 in QGP-1. Both mutations were validated through Sanger sequencing and predicted to be damaging by all four prediction algorithms. The predicted loss of function of p53 in the cell lines is supported by the previously reported lack of p53 mRNA and protein expression detectable by immunohistochemistry (Arany et al. 1994, Bartz et al. 1996). These findings imply that the two cell lines, at least partially, might rely on inactivation of p53 to escape cell death.

**Mutations in ATRX, RAS, and PI3K–AKT–mammalian target of rapamycin pathway genes**

Recent exome-sequencing efforts on tumor material from sporadic PNET patients have detected frequent mutations in ATRX, DAXX, and MEN1 (Jiao et al. 2011). ATRX and DAXX form a complex that plays a role in the incorporation of histone variant H3.3 at the telomeres, acting as epigenetic regulators (Lewis et al. 2010). MEN1 encodes menin, a nuclear protein important in chromatin remodeling and gene expression through histone acetylation and deacetylation (Kim et al. 2003). Conflicting results on the effects of ATRX/DAXX alterations have been obtained. In one study, ATRX/DAXX mutations correlated with a better survival in PNET patients with metastatic disease (Jiao et al. 2011). In other studies, with mainly early-stage-disease patients, loss of ATRX/DAXX expression was associated with a worse prognosis (Marinoni et al. 2014, Yuan et al. 2014). In BON-1, the ATRX gene contains a homozygous (AF 100%) g.77682471C>G missense mutation (ENSP00000362441 p.Q929E) and in QGP-1 a homozygous (AF 100%) g.77682716A>G missense mutation (ENSP00000362441 p.F847S) was seen. The real effects of the two mutations on protein function are unknown, but all algorithms predicted them both to be benign. There were no mutations of the DAXX gene in BON-1 and QGP-1, confirming the mutually exclusive nature of DAXX/ATRX mutations. Further functional studies are necessary to elucidate the role of the ATRX/DAXX pathway in BON-1 and QGP-1. Although in previous studies no MEN1 mutation was identified in BON-1 (Stalberg et al. 2004), our results revealed a missense homozygous (AF 100%) g.64572018T>C MEN1 mutation.

### Table 2: SNVs confirmed by Sanger sequencing in BON-1 and QGP-1

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Location</th>
<th>Reference allele</th>
<th>Alternative allele</th>
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SNP (ENSP00000377899 p.T546A) in both BON-1 and QGP-1. However, this SNP is benign according to the ClinVar database and thus probably does not play a role in the malign transformation of the BON-1 and QGP-1 cells (Landrum et al. 2014).

In a previous study, a homozygous mutation in NRAS codon 61 was identified in BON-1 (Arany et al. 1994). In our BON-1 data, a homozygous (AF 100%) g.114713908T>C mutation (ENSP0000038548 p.Q61R) was found in codon 61 of NRAS. In addition, a heterozygous g.25245350C>A mutation (ENSP0000038548 p.Q61V) with an allelic ratio of 0.71 was found in the KRAS gene in QGP-1. Both the NRAS mutation in BON-1 and the KRAS mutation in QGP-1 were predicted to be damaging by all four prediction algorithms used. Results from a recent study have indicated that KRAS mutations are present in 10% of Chinese PNET patients and correlate with a poorer prognosis (Yuan et al. 2014). This might indicate a role of the RAS pathway in the oncogenesis of PNET.

The phosphoinositide-3-kinase/AKT/mammalian target of rapamycin (PI3K–AKT–mTOR) signaling pathway has been demonstrated to play a major role in NET by regulating cell growth, proliferation, survival, and protein synthesis (Kasajima et al. 2011). Hence, mTOR-inhibiting rapamycin and analogs (rapalogs) such as everolimus have a role in NET therapy. Results from a phase III trial were indicative of improved progression-free survival with everolimus as mono-therapy in progressive advanced PNET (Yao et al. 2011). However, in 15% of sporadic PNET mutations were found in PI3K–AKT–mTOR pathway-related genes such as PTEN, PI3KCA, and TSC2 (Jiao et al. 2011). No mutations were detected in the PI3K–AKT–mTOR pathway genes PTEN and PI3KCA in BON-1 and QGP-1. However, three missense mutations were detected in TSC2 in BON-1, including a g.2083797G>A mutation (AF 0.63; ENSP0000344383 p.R1306H) predicted to be damaging by SIFT. This pattern of mutations could be indicative of a role of the TSC2 and, thus, the PI3K–AKT–mTOR pathway in the neoplastic properties of the BON-1 cell line. In addition, comparing BON-1, a cell line with a TSC2 gene mutation, and QGP-1, a cell line without mutations in the PI3K–AKT–mTOR pathway, could elucidate the importance of these mutations in the efficacy of mTOR-directed drugs. Having a model for PNET with and without PI3K–AKT–mTOR pathway mutations could also be helpful for drug-development of targeted agents.

Mucin as a possible new biomarker in PNET

Mucins are high-molecular-weight glycoproteins with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by O-glycosidic linkages that are produced by various epithelial cells and play a role in cell–cell interactions (Yonezawa et al. 2008). MUC genes are highly variable in humans and often contain novel and unique SNVs in germline samples. For this reason, MUC mutations are sometimes removed from further analysis in studies of NGS data. However, mucin overexpression has frequently been seen in PNET tumor samples (Carrara et al. 2011, Carr et al. 2013). Hence, this study looked into the presence and relevance of MUC gene alterations in BON-1 and QGP-1. MUC4, MUC6, MUC16, and MUC17 genes showed possible protein-altering mutations in both cell lines. In addition, the MUC2, MUC5B, MUC12, and MUC13 genes show mutations with possible effects on proteins in BON-1. Further research is needed to fully understand the mechanisms that underlie the involvement of mucins in PNET.

In conclusion, the first, to our knowledge, high-resolution genetic profile of the BON-1 and QGP-1 cell lines, based on array-based techniques and next generation whole-exome sequencing data, is presented in this article, greatly expanding on previous low-resolution genomic data for BON-1 and QGP-1 published previously. These frequently used PNET models show mutations in accordance with published findings regarding sporadic PNET in patients. However, further validation of BON-1 and QGP-1 as relevant genetic models for different subtypes of PNET in patients is needed. We believe that our data could contribute to a better understanding of the pathogenesis of PNET.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JME-14-0304.

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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References


