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Short communication

Identification of nonsynonymous *TP53* mutations in hydatidiform moles

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ABSTRACT

Hydatidiform mole (HM), an unusual pregnancy with pure or predominant paternal genetic contribution, is the most common form of gestational trophoblastic disease. Most HM regress after uterine evacuation but some will develop into persistent disease or even frank malignancy. Although p53 is highly expressed in HM, *TP53* mutations have rarely been detected in previous studies. Here we screened for specific missense mutations on several *TP53* hotspots in 49 HMs using a highly sensitive pyrosequencing approach and revealed the significant existence of such mutations in HM tissues. A particularly high frequency (~59% of the cases) of p53 inactivating mutation on exon 7 has been detected. Our identification of hitherto unreported *TP53* mutations in HM suggests the presence of p53 mutants and reflects the advantages of using pyrosequencing for point mutation detection in clinical samples. Traditional sequencing method may have overlooked such mutations that only occur in a small population of trophoblasts.

1. Introduction

Gestational trophoblastic disease (GTD) is a heterogeneous disease of placental trophoblasts with clinically distinct pathophysiology. It includes benign hydatidiform mole (HM) that carries malignant potential to frankly malignant choriocarcinoma, placental site trophoblastic tumour and epithelioid trophoblastic tumour [1]. HM often presented with symptoms and signs of missed abortion and majority of HM will regress after suction evacuation. About 8–30% of patients with HM will progress to persistent GTD or even choriocarcinoma and require chemotherapy although most of them are curable [2]. Two forms of molar pregnancy, complete and partial HM, are of distinctly clinical, pathological and genetic features. Complete HM, which is usually diploid with pure androgenetic karyotype (46,XX; 46,XY), and partial HM, which is often triploid resulting from fertilisation of an ovum by two sperms (usually 69,XXY or XXX), are the two forms of HM [3].

*TP*53 is one of the most commonly mutated genes in human malignancies and is considered to be an important tumour suppression gene. Extensive studies have demonstrated a wide variety of mutations spreading across this gene, whereas some patterns of *TP*53 mutations occur with a particularly high frequency in certain malignancies that can be recognised as a diagnostic feature [4]. We have reported a higher expression of p53 in HM and choriocarcinoma than normal placenta [5]. Other studies also detected higher expression of p53 mutant in HM through immunohistochemistry [6] and high p53 mutant expression was associated with the progression to aggressive disease [7]. Nevertheless, genetic analysis on *TP53* mutation done by us and other groups have shown absence or occasional *TP53* mutations in HM [5,8,9]. Such contradictory findings may be due to the limited sensitivity of sequencing approach used in previous studies. A mutation that only occurs in a small subpopulation of cells may not be demonstrable by Sanger sequencing [10].

Pyrosequencing is a sensitive method for analysis of DNA sequences of short read length and individual base at precise position [11]. Therefore, it is useful for identifying point mutation and single nucleotide polymorphism of low incidence within a sample of heterogeneous population. So far, investigation on *TP53* mutations on HM is mostly based on Sanger sequencing [5,8,9]. In this study, nine specific point mutations spanning across the hotspots located on exon 5 to exon 8, which were based on the International Agency for Research on Cancer (IARC) *TP53* database (http://p53.iarc.fr), have been assessed with pyrosequencing in HM samples.

2. Materials and methods

2.1. Clinical samples

A total of 49 frozen tissue blocks, including 43 complete and 6

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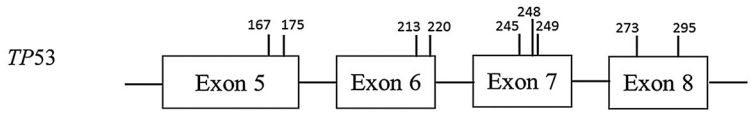
A



Exon-codon	Forward primer	Reverse primer	Sequencing primer	Expected size (bp)
5-167,175	Biotin-CGCGCCATGGCCATCTAC	ATGCTGAGGAGGGGCCAGA	1. CCTCACAACCTCCGT 2. AGCAGCGCTCATGGT	108
6-213,220	TGCGTGTGGAGTATTTGGATGA	Biotin-TGGTACAGTCAGAGCCAACCTCAG	TGGATGACAGAAACACTT	90
7-245,248,249	CCTATGAGCCGCCTGAGGT	Biotin-GCTGTTCCGTCCCAGTAGATTACC	AACAGTTCCTGCATGG	152
8-273,295	AATCTACTGGGACGGAACAGC	Biotin-TGGGCAGTGCTCGCTTAG	1. ACGGAACAGCTTTGA 2. TCTCCGCAAGAAAGG	142

B

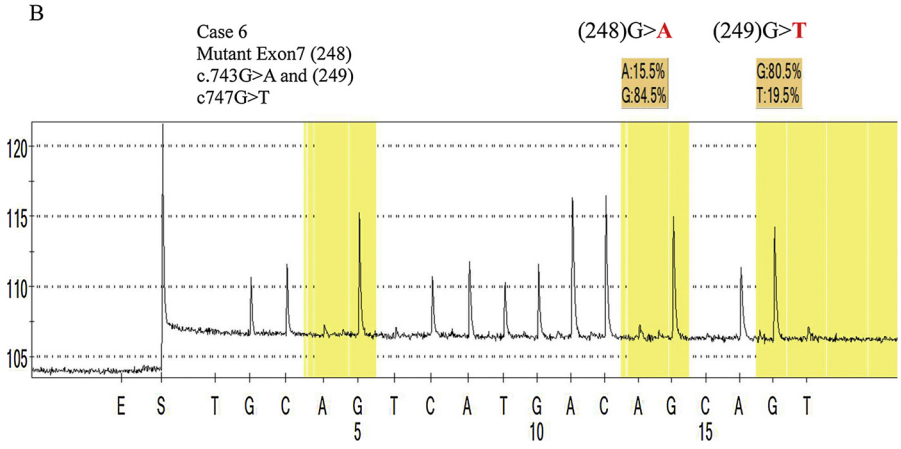


Fig. 1. A) Distribution of the mutation sites on *TP53* being screened are shown. The numbers above denotes the codons on each exon. The primers for each exon and the expected product size are shown. B) The representative pyrogram of an individual HM case is shown to illustrate the missense mutations on codon 248 and codon 249 of exon 7. The bases being replaced in mutant forms are highlighted and bolded. The percentage indicates the fraction of cDNA harbouring the base in that sample.

partial HM, were retrieved. These patients were treated between 1990 and 2002 at Queen Mary Hospital and the histological features were assessed based on standard diagnostic criteria. Thirty three cases spontaneously regressed after suction evacuation and 16 cases progressed to persistent disease requiring chemotherapy with metastasis detected in six cases. The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

2.2. Synthesis of cDNA and pyrosequencing for TP53

RNA was extracted using TRIzol reagent solution (Ambion, Massachusetts, USA). Reverse transcription and subsequent PCR amplification was carried out by PyroMark OneStep RT-PCR Kit (Qiagen, Hilden, Germany). Nine point mutations spanning across exon 5–8 were examined. The primers for those amplicons were shown on Fig. 1A. The RT-PCR conditions were used according to the manufacture's instruction with modification. The amplification condition for exon 5: 50 °C 30 min, 95 °C 15 min, [94 °C 30 s, 60 °C 30 s, 72 °C 30 s], 72 °C 10 min for 45 cycles; exon 6: 50 °C 30 min, 95 °C 15 min, [94 °C 30 s, 55 °C 30 s, 72 °C 30 s], 72 °C 10 min for 45 cycles; exon 7: 60 °C 30 min, 95 °C 15 min, [94 °C 30 s, 62 °C 30 s, 72 °C 30 s], 72 °C 10 min for 45 cycles; exon 8: 45 °C 30 min, 95 °C 15 min, [94 °C 30 s, 50 °C 30 s, 72 °C 30 s], 72 °C 10 min for 45 cycles. The success of PCR was confirmed with electrophoresis on agarose gel. *TP53* pyrosequencing was performed using PyroMark Q96 MA platform with the support from Centre for Genomic Sciences, University of Hong Kong.

2.3. Statistical analysis

With the Crosstab analysis using SPSS version 24 (SPSS Inc., Chicago, IL), the interrelation between a point mutation and the clinical outcome was analysed. Pearson correlation score was also acquired to justify the significance of these associations.

3. Results

In this study, 9 specific missense and nonsense mutation hotspots spanning across exon 5 to exon 8 were assessed with pyrosequencing on 49 HM samples. In general, 36 out of 49 cases (73.47%) harboured at least one *TP53* mutation being analysed. Among these mutations, c.747G > T of codon 249 (Exon7) exhibited the highest frequency. It was found in 27 out of 43 (62.79%) complete HM and 2 out of 6 (33.33%) partial HM. The overall rate of c.747G > T in HM was hence 59.18%. Such nonsynonymous substitution results in arginine to serine conversion at codon 249 (p.R249S). Another missense mutation c.743G > A was observed in exon 7 codon 248 (CGG > CAG) where an arginine is substituted with glutamine (p.R248Q). c.743G > A was found in 8 out of 43 (18.60%) complete HM and 1 out of 6 (16.67%) partial HM. The overall frequency in HM was 18.37%. All the cases with p.R248Q mutation were accompanied with p.R249S but not the vice versa. Notably, a nonsense mutation c.637C > T (CGA > TGA, p.R213X) in exon 6 on codon 213 [12] occurred in 15 out of 43 (34.88%) complete HM and 2 out of 6 (33.33%) partial HM. The overall frequency was about 35%. The percentage of mutation detected in individual case was shown in Table 1A. The mutation frequency in cases with or without neoplastic progression was also shown but none of these mutations showed significant correlation with progression to neoplasia (Table 1B). Representative pyrograms of codons with mutations were also shown (Suppl. Fig. 1A). Notably, none of these point mutations were observed in a normal healthy donor and a first trimester placenta sample (Suppl. Fig. 1B), strongly suggesting that these