

SHORT COMMUNICATION

Lack of Rb2/p130 genetic alteration in Malaysian nasopharyngeal carcinoma

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Abstract

The retinoblastoma-related gene Rb2/p130 has been reported to be mutated in several malignancies such as lung cancer and Burkitt's lymphoma. Nasopharyngeal carcinoma (NPC) is a common cancer in Malaysia especially amongst the ethnic Chinese. We screened for Rb2/p130 gene (exons 19 to 21) mutations in 53 archival NPC samples via PCR-SSCP-direct sequencing approach. Only one sample had a base change which involved a serine to glycine substitution at codon 995 (S995G). We conclude that Rb2/p130 genetic alterations are infrequent in NPC and may not be essential for the pathogenesis of the disease.

Key words: Rb2/p130 gene, nasopharyngeal carcinoma, PCR-SSCP

INTRODUCTION

The retinoblastoma-related gene Rb2/p130 is a member of the retinoblastoma (Rb) gene family. Despite sharing homologies with the other members of the Rb family, namely Rb/p105 and p107, Rb2/p130 is reported to be most crucial for the control of cell cycle progression.¹ It consists of 22 exons spanning more than 50kb of genomic DNA with a pocket region made up of domains A and B. Most of Rb2/p130 genetic alterations found in lung carcinoma and Burkitt's lymphomas are found within its pocket domain B and C-terminus.^{2,3}

Nasopharyngeal carcinoma (NPC) remains a common cancer in Malaysia, especially among the Chinese males. Although susceptibility factors such as dietary carcinogens, certain HLA polymorphisms and the presence of the Epstein-Barr virus have been associated with the malignancy, identification of genes frequently mutated remain useful for elucidating the mechanisms of nasopharyngeal carcinogenesis and for discovering new molecular targets for its diagnosis, treatment or prevention. One report found that Rb2/p130 mutation incidence was high in NPC but others suggested that the frequency of mutation differ based on geographical origins.^{4,5}

METHODOLOGY AND RESULTS

We performed a mutational screening of the Rb2/p130 gene on 53 formalin-fixed, paraffin-embedded NPC samples from our local population. Routine haematoxylin and eosin (H&E) as well as immunohistochemically stained slides of these tissue blocks were reviewed and reconfirmed as NPC by a pathologist (SCP). The samples were amplified and screened for mutation by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method in exons 19 to 21. The region was analyzed due to its importance in mediating the pocket region's binding to viral and cellular proteins and, the reported presence of majority of Rb2/p130 gene mutations occurring here.^{3,4} Samples displaying mobility band shift in the SSCP polyacrylamide gels were sequenced to confirm the genetic alteration(s). Genomic DNA extracted from human placenta and healthy individual sample were used as negative controls.

All the exons were successfully amplified in the NPC samples. Eight samples showing apparent mobility shift were re-amplified and sequenced (Fig. 1). Only one sample (T71) showed an alteration: a heterozygous A>G

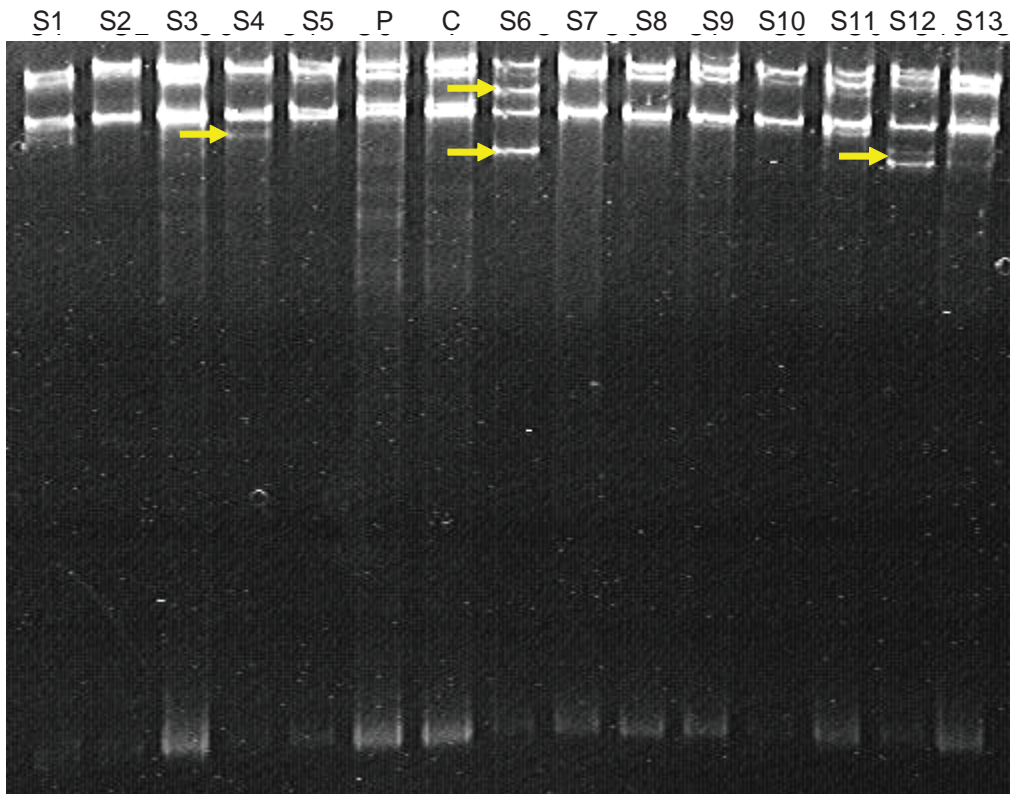


FIG. 1. SSCP analysis of Rb2/p130 exon 20. S4, S6, S12, patients' tumour samples with a mobility shift as indicated by arrow; S1-S3, S5, S7-S11, S13, patients' tumour samples; P, human placenta; C, healthy individual sample.

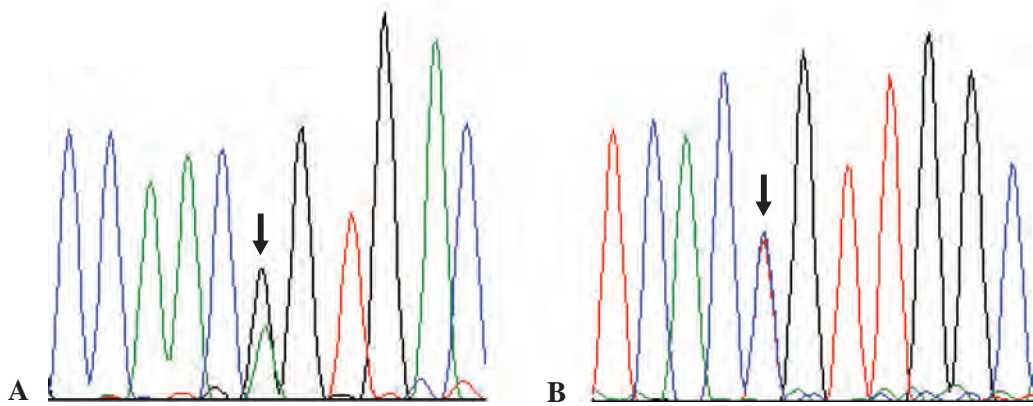


FIG. 2. Sequence analysis of Rb2/p130 exon 20 for sample T71 shows an A>G heterozygous base change. A. Forward strand. B. Reverse strand.

nucleotide change at codon 995 in exon 20 (Fig. 2). The alteration was predicted to cause a serine to glycine amino acid substitution (S995G). In order to rule out polymorphism, 50 blood DNA from healthy individuals were amplified and sequenced. All the blood DNA did not show sequence alteration.

DISCUSSION

Most of the commonly reported Rb2/p130 gene mutations were insertions and/or point mutations which led to frameshifts and amino acid changes, respectively. A Kenyan study found that majority of their endemic Burkitt's lymphoma (BL) samples had mutations in exons 19 to 21 as compared to wild type Rb2/p130 gene presence in sporadic BL and AIDS-related BL samples.³ Claudio *et al.* reported 30% of their African NPC biopsies had insertions in exons 19 and 21.⁴ Further studies on a larger set of African NPC samples with the inclusion of some Chinese NPC samples also revealed similar percentage of Rb2/p130 mutations in the Africans as compared to wild-type Rb2/p130 in the Chinese.⁶ Less than 2% of our Malaysian NPC samples had a sequence alteration. Similar PCR-SSCP-sequencing methodology used in ovarian and lung carcinomas, sporadic BL tumours, glioblastomas and adult T-cell leukaemia/lymphoma had also revealed no or rare Rb2/p130 mutation occurrence.⁷⁻¹¹

The A>G nucleotide change at codon 995 in our study may be a true heterozygous mutation or it may be due to the patient's mixture of normal genomic DNA from infiltrating lymphocytes together with NPC cells with homozygous mutation. We could not confirm it as a result of the lack of the patient's normal tissue block. Codon 995 is within the pocket domain of the Rb2/p130 gene but not within known nuclear localization signal (NLS) sequences.¹² Although correct NLS sequences are crucial for proper translocation of the protein to the nucleus, an intact pocket domain also plays a defining role in nuclear translocation.¹² Thus, the amino acid substitution in sample T71 could potentially disrupt the export of Rb2 protein to the nucleus. In addition, proper binding of Rb2 to E2F transcription factors which are vital to cell cycle control may have been disturbed by the alteration. Further work needs to be done in order to fully characterize this particular alteration. As only one out of our 53 NPC samples had a genetic change, we conclude that mutations in exons

19 to 21 of the Rb2/p130 gene are unlikely to be important for the pathogenesis of most NPC cases in Malaysia.

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REFERENCES

1. D'Andrilli G, Masciullo V, Bagella L, et al. Frequent loss of pRb2/p130 in human ovarian carcinoma. *Clin Cancer Res.* 2004; 10: 3098-103.
2. Claudio PP, Howard CM, Pacilio C, et al. Mutations in the retinoblastoma-related gene RB2/p130 in lung tumors and suppression of tumor growth in vivo by retrovirus-mediated gene transfer. *Cancer Res.* 2000; 60: 372-82.
3. Cinti C, Leoncini L, Nyong'o A, et al. Genetic alterations of the retinoblastoma-related gene RB2/p130 identify different pathogenetic mechanisms in and among Burkitt's lymphoma subtypes. *Am J Pathol.* 2000; 156: 751-60.
4. Claudio PP, Howard CM, Fu Y, et al. Mutations in the retinoblastoma-related gene RB2/p130 in primary nasopharyngeal carcinoma. *Cancer Res.* 2000; 60: 8-12.
5. Gray SG, Guo X, Kedra D, Teh BT, Min HQ. Correspondence re: P. P. Claudio et al., Mutations in the retinoblastoma-related gene RB2/p130 in primary nasopharyngeal carcinoma. *Cancer Res.* 60: 8-12, 2000. *Cancer Res.* 2001; 61: 5950-1.
6. Claudio PP, Cinti C, Paggi MG, Giordano A. Correspondence re: Gray SG et al., Mutations in the retinoblastoma-related gene RB2/p130 in primary nasopharyngeal carcinoma. *Cancer Res.*, 60: 8-12, 2000. *Cancer Res.* 2001; 61: 5952-3.
7. Alvi AJ, Hogg R, Rader JS, Kuo MJ, Maher ER, Latif F. Mutation screening analysis of the retinoblastoma related gene RB2/p130 in sporadic ovarian cancer and head and neck squamous cell cancer. *Mol Pathol.* 2002; 55: 153-5.
8. Xue Jun H, Gemma A, Hosoya Y, et al. Reduced transcription of the RB2/p130 gene in human lung cancer. *Mol Carcinog.* 2003; 38: 124-9.
9. Klumb CE, Magluta EP, Rezende LM, Apa AG, Alonso JF, Maia RC. Retinoblastoma-related gene Rb2/p130 are rarely mutated in Burkitt's lymphoma from Brazil. *Am J Hematol.* 2007; 82: 238-9.
10. Gonzalez-Gomez P, Bello MJ, Arjona D, et al. Retinoblastoma-related gene RB2/p130 exons 19-

- 22 are rarely mutated in glioblastomas. *Oncol Rep.* 2002; 9: 951-4.
11. Takeuchi S, Takeuchi N, Tsukasaki K, et al. Mutations in the retinoblastoma-related gene RB2/p130 in adult T-cell leukaemia/lymphoma. *Leuk Lymphoma.* 2003; 44: 699-701.
 12. Chestukhin A, Litovchick L, Rudich K, DeCaprio JA. Nucleocytoplasmic shuttling of p130/RBL2: novel regulatory mechanism. *Mol Cell Biol.* 2002; 22: 453-68.