Pathol. Oncol. Res. (2008) 14:239–241
DOI 10.1007/s12253-008-9024-1

ORIGINAL PAPER

Systemic Dissemination in Cancer of Unknown Primary is Independent of Mutational Inactivation of the KiSS-1 Metastasis-suppressor Gene

L. Dova · V. Golfinopoulos · G. Pentheroudakis · I. Georgiou · N. Pavlidis

Received: 24 August 2007 / Accepted: 30 October 2007 / Published online: 20 March 2008

Abstract Cancer of unknown primary represents a heterogeneous group of malignancies characterised by early systemic dissemination and lack of primary site. KiSS1 is a member of the metastasis-suppressor gene family whose functional role is being investigated in human malignancies. We extracted DNA from 50 paraffin-embedded unknown primary tumors and screened KiSS1 exons III and IV for presence of mutations by means of Single Strand Conformational Polymorphism and direct sequencing. Only one tumor specimen harboured a cytosine to guanine point substitution in base 242 of exon IVa, resulting in a proline to arginine switch at codon 81 of the KiSS1 protein (P81R). The remaining 49 tumors harbored wild-type KiSS1 alleles, indistinguishable from those of peripheral blood lymphocytes of 50 healthy controls. Consequently, the propensity for systemic spread of unknown primary tumors may by due to mutations in genes other than KiSS1 or aberrant epigenetic regulation.

L. Dova
Molecular Biology Unit, Hematological Laboratory,
Ioannina University Hospital,
Ioannina, Greece

V. Golfinopoulos · G. Pentheroudakis · N. Pavlidis
Department of Medical Oncology, Ioannina University Hospital,
Ioannina, Greece

I. Georgiou
Genetics Unit, Department of Obstetrics and Gynecology,
Ioannina University Hospital,
Ioannina, Greece

N. Pavlidis (✉)
Medical School, University of Ioannina,
Niarxou Avenue,
45500 Ioannina, Greece
e-mail: npavlid@cc.uoi.gr

Keywords CUP · KiSS-1 metastasis-suppressor gene · Polymerase chain reaction · KiSS-1 · FFPE

Introduction

Cancer of unknown primary site (CUP) is a heterogeneous group of tumors that have the propensity to metastasize early, leading to clinical recognition of metastases without a demonstrable primary tumor. The KiSS-1 metastasis-suppressor gene consists of four exons (I–IV) and is located in chromosome 1q32 [1]. Only exons III and IV are transcribed and encode a 145 amino acid hydrophilic protein (Metastin or Kisspeptin) that has been shown to exert metastasis-suppressing effects after transfection into human breast carcinoma and melanoma cell lines [2, 3]. The study for KiSS-1 mutations in widely and early disseminating tumors, such as cancers of unknown primary, is rationally based on hypothesis-generating preclinical data.

Materials and Methods

We collected formalin-fixed paraffin embedded biopsy specimens from 50 patients with cancer of unknown primary site from 2002 until 2005 and obtained 10-μm thick sections containing at least 75% malignant cells as judged by hematoxylin-eosin staining for DNA isolation and mutational analyses of exons III, IVa and IVb (exon IV was spliced in two fragments because of its large size). Genomic DNA was also extracted from normal peripheral blood lymphocytes of 50 healthy controls.

Polymerase chain reaction (PCR) amplification of exons III and IV was performed in a total volume of 50 μl containing 50 ng of genomic DNA, dNTP, Taq DNA

Fig. 1 KiSS1 exon IVa SSCP aberrant band on polyacrylamide gel electrophoresis. *From left to right*: *lane 1*—negative control, *lanes 2 to 5*—CUP specimens (*lane 5* bears the 242 C > G mutation), *lane 6*—positive control

Polymerase and each of the following primers under standard thermal cycling conditions:

Exon III—Forward 5′-CTC AGC CTC AAG GCA CTT CT-3′, Reverse 5′-CAC TCC TTT CCC CAG AGG AT-3′, Exon IVa—Forward 5′-TCC TAG GCC AGC AGC TAG AA-3′, Reverse 5′-CCA GTT GTA GTT CGG CAG GT-3′, Exon IVb—Forward 5′-ACC TGC CGA ACT ACA ACT GG-3′, Reverse 5′-TCT TTT ATT GCC TCG GGT TG-3′.

Mutational screening was performed by means of Single Strand Conformational Polymorphism (SSCP) of amplified PCR products in a denaturing loading buffer followed by polyacrylamide gel electrophoresis and sequencing analysis (MWG The Genomic Company, Ebersberg Germany).

Results

We were unable to observe exon III or IVb point mutations, insertions or deletions in any of the 50 CUP samples nor in lymphocytes of 50 healthy controls. Moreover, sequencing of the SSCP bands yielded wild-type exon III and IVb motifs. A shifted exon IVa SSCP band was seen in one of 50 CUP samples, harbouring a cytosine to guanine substitution 242 base pairs from the translation start site (242 C to G) and resulting in substitution of the amino-acid proline by arginine (P81R) (Figs. 1 and 2).

Discussion

Solid tumor metastasis seems to be regulated by the interplay of metastasis-promoter and metastasis-suppressor genes among which KiSS1 is the only one that binds a G-protein coupled receptor [1, 2]. In vitro data identifying KiSS1 as a putative metastasis suppressor were corroborated by clinical series of patients with solid tumors that associated low KiSS1 mRNA or protein expression with advanced clinical stage, metastases and relapse [3]. Recently, we discovered a novel mutation in exon IVa of the *KiSS-1* gene in early-stage breast adenocarcinomas harboured by 17 out of 50 women [4]. This 242 C to G point mutation results in substitution of the hydrophobic amino-acid proline by the polar, hydrophilic amino-acid arginine (P81R). This is likely to result in modification of the tertiary stereotactic structure and thus, function, of the KiSS-1 protein. The lack of KiSS1 mutations in this CUP cohort does not establish a central role for mutational inactivation of this metastasis-suppressor gene in cancer of unknown primary site, though it does not rule out epigenetic suppression of gene transcription neither post-translational protein modifications. In view of the number of genes modulating the cancer cell's metastatic phenotype,

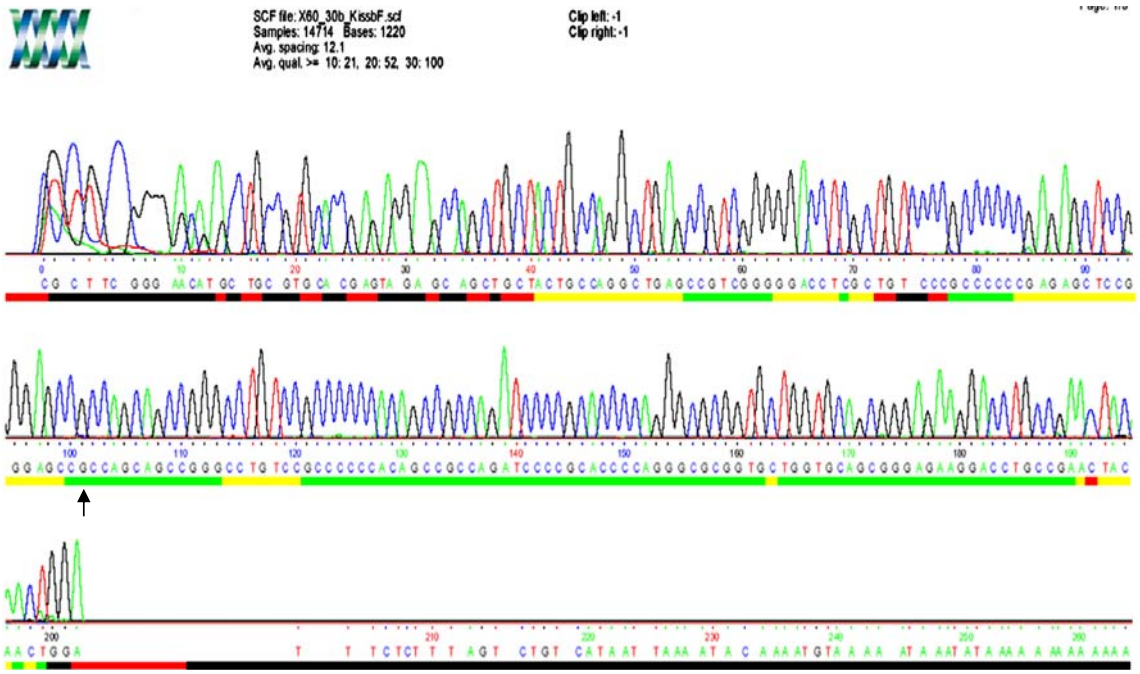

Fig. 2 KiSS1 exon IVa mutated sequence (*position 101*, *arrow*)

a regular, pro-metastatic pattern of genetic changes in a relatively small set of genes may not exist in CUP [5]. Combined multigene expression analysis using microarray technology may represent a better approach to the study of the genetic characteristics of this type of tumor.

References

1. Yoshida BA, Sokoloff M, Welch DR, Rinker-Schaeffer CW (2000) Metastasis-suppressor genes: a review and perspective on an emerging field. J Natl Cancer Inst 92:1717–1730
2. West A, Vojta PJ, Welch DR, Weissman BE (1998) Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). Genomics 54:145–148
3. Nash KT, Welch DR (2006) The KISS1 metastasis suppressor: mechanistic insights and clinical utility. Front Biosci 11:647–659
4. Pentheroudakis G, Kostadima L, Dova L, Malamou-Mitsi V, Georgiou I et al (2006) The missing kiss of life: Analysis of mutation and protein expression of the metastasis-suppressor gene KISS1 in early breast cancer. Ann Oncol 17(Suppl 9):ix59 (101P)
5. Pentheroudakis G, Briasoulis E, Pavlidis N (2007) Cancer of unknown primary site: missing primary or missing biology? Oncologist 12:418–425