Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
J. Biochem. Biophys. Methods 63 (2005) 222–227
JOURNAL OF biochemical and biophysical methods
www.elsevier.com/locate/jbbm

Short note

DHPLC analysis of the matrix metalloproteinase-1 promoter 1G/2G polymorphism that can be easily used to screen large population

Enping Xu, Maode Lai*, Bingjian Lv, Xiaoming Xing, Qiong Huang, Yu Ma, Wei Wang

Department of Pathology and Pathophysiology, Center for Environmental Genomics, School of Medicine, Zhejiang University, Hangzhou 310031, China

Received 9 October 2004; received in revised form 17 March 2005; accepted 21 March 2005

Abstract

Matrix metalloproteinase-1 has been shown to play an important role in all stages of cancer initiation, invasion, and metastasis. The 1G/2G single nucleotide polymorphism (SNP) at −1607 to −1608 creates an Ets binding site and elevates the rate of transcription. Moreover, the presence of the 2G allele in the MMP-1 promoter has been reported to be associated with the development and/or progression of carcinomas of the ovary, endometrium, lung, and colorectum. However, further studies on a wide variety of cancers in various sufficiently large populations will be required to verify that 2G is risk factor for cancers. A major challenge confronting such studies is the need to develop accurate, fast and inexpensive high-throughput genotyping techniques. To set up a fast and sensitive test for MMP-1 1G/2G genotyping, we analyzed 126 healthy persons by denaturing high performance liquid chromatography (DHPLC). The genotypes of MMP-1 1G/2G revealed by DHPLC analysis were further confirmed by DNA sequencing. In conclusion, DHPLC is a cost-effective, rapid, sensitive, and high-throughput technique for MMP-1 1G/2G genotyping.

Keywords: DHPLC; Matrix metalloproteinase-1; Single nucleotide polymorphism

* Corresponding author. Fax: +86 571 87217497.
E-mail address: lmd@zju.edu.cn (M. Lai).

0165-022X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbbm.2005.03.007

1. Introduction

The matrix metalloproteinases (MMPs) comprise a family of at least 20 proteolytic enzymes that are capable of selectively degrading a wide spectrum of extracelluar matrix proteins [1]. Accumulating evidence has shown that over-expression of metalloproteinases is associated with tumor development, invasion, metastasis, and worse prognosis [2–4]. MMP-1 degrades fibrillar collagens, the most abundant class of extracelluar matrix proteins in interstitial connective tissue. Numerous studies have demonstrated that MMP-1 plays an essential role in promoting tumor invasiveness and metastasis [5,6]. Rutter et al. [7] have reported the −1607 to −1608 insG in the MMP-1 promoter, creating an Ets binding site 5′-GGA-3′. Promoters containing this 2G sequence have significantly higher transcription rate in normal fibroblasts and in melanoma cells than 1G promoter. Moreover, the presence of the 2G allele in the MMP-1 promoter has been reported to be associated with the development and/or progression of carcinomas of the ovary, endometrium, lung, and colorectum [4,8–11]. Thus, this MMP-1 genetic polymorphism is a promising biomarker for cancer and prognosis. In this regard, screening for the MMP-1 1G/2G polymorphism in a large population would be of great interest. However, some traditional methods for genotyping this polymorphism are time-consuming, expensive, non-automatic, or radioactive. In contrast, DHPLC – which enables the rapid, sensitive, and accurate identification of polymorphisms and mutations in an automated fashion – was used for MMP-1 1G/2G genotyping in this study. DHPLC is a robust technique that can be used to screen any gene in a large population for single nucleotide substitutions, as well as small deletions and insertions. The technique has been described elsewhere [12,13]. Briefly, polymorphisms and mutations scanned by DHPLC involve subjecting polymerase chain reaction (PCR) products to ion-pair reverse phase liquid chromatography in a column containing alkylated nonporous particles. Non-denaturing analysis of PCR products at 50 °C demonstrates product size, purity, and yield. Under conditions of partial heat denaturation within a linear acetonitrile gradient, heteroduplexes that form in PCR samples having internal sequence variation display reduced column retention time relative to their homoduplex counterparts.

2. Materials and methods

2.1. Study subjects

A cohort of 126 healthy persons was obtained from patients at affiliated hospitals, Zhejiang University College of Medicine (Hangzhou, China). Genomic DNA was prepared by digestion with proteinase K and phenol-chloroform extraction from the peripheral blood.

2.2. PCR

PCR was carried out in a total volume of 25 μl containing ~100 ng of DNA, 0.25 μM forward and reverse primers (5′–CCCTC TTGAA CTCAC ATGTT ATG–3′ and 5′–