Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 254 (2007) 42–53
CANCER Letters
www.elsevier.com/locate/canlet

Differential expression of angiogenesis-related genes in human gastric cancers with and those without high-frequency microsatellite instability

Nobuki Miyamoto [a,1], Hiroyuki Yamamoto [a,*,1], Hiroaki Taniguchi [a], Chie Miyamoto [a], Mariko Oki [a], Yasushi Adachi [a], Kohzoh Imai [b], Yasuhisa Shinomura [a]

[a] *First Department of Internal Medicine, Sapporo Medical University, South-1, West-16, Sapporo 060-8543, Japan*
[b] *Sapporo Medical University, South-1, West-17, Sapporo 060-8557, Japan*

Received 4 September 2006; received in revised form 16 November 2006; accepted 5 February 2007

Abstract

Gastric cancers with and those without high-frequency microsatellite instability (MSI-H) represent distinctive pathways of carcinogenesis. The aim of this study was to clarify if expression of p53 related genes involved in angiogenesis is differentially regulated between these cancers. We systematically analyzed the expression of vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), thrombospondin 1 (THBS1), and brain-specific angiogenesis inhibitor 1 (BAI1), and we correlated the results with microvessel count (MVC), MSI status, p53 mutations, and prostaglandin-endoperoxide synthase 2 (PTGS2) expression in gastric cancers. Expression of VEGFA in carcinoma cells was immunohistochemically seen in 46% of 200 cases. VEGFA positivity was significantly associated with higher MVC, vascular invasion, lymph node and distant metastasis, and advanced tumor stage. FGF2 positivity was significantly associated with poor differentiation, depth of invasion, and higher MVC. VEGFA and FGF2 positivities and MVC were lower in MSI-H cancers than in MSI-L or MSS cancers. VEGFA expression was associated with both p53 mutations and PTGS2 expression. Methylation of the THBS1 gene was detected in 6 of 11 cancer cell lines and in 44% of 200 cases. THBS1 methylation was significantly associated with distal location, vascular invasion, distant metastasis, MSI-H, wild-type p53, and higher MVC. The prognosis was worst in patients with cancers that were VEGFA-positive and THBS1 methylation-positive. Gastric cancers with MSI-H were characterized by lower MVC, low frequency of VEGFA, FGF2, and PTGS2 overexpression, and high frequency of THBS1 methylation. Our results suggest that gastric cancers with and those without MSI-H represent distinctive pathways of carcinogenesis, including aberrant expression of factors regulating angiogenesis. The difference may be associated with less aggressive phenotype of these cancers with MSI-H and affect future molecular targeted therapeutics.

Keywords: Microsatellite instability; Angiogenesis; Gastric cancer; VEGF; Thrombospondin 1

* Corresponding author. Tel.: +81 11 611 2111x3211; fax: +81 11 611 2282.
E-mail address: h-yama@sapmed.ac.jp (H. Yamamoto).
[1] These authors contributed equally to this manuscript.

0304-3835/$ - see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2007.02.004

1. Introduction

A type of genetic instability characterized by length alterations within simple repeated sequences, termed high-frequency microsatellite instability (MSI-H), occurs in the majority of hereditary non-polyposis colorectal cancers (Lynch syndrome) and in a subset of sporadic cancers [1–3]. Colorectal cancers with and those without MSI-H exhibit fundamental differences in clinical, pathological, and molecular characteristics [1–5]. Expression of vascular endothelial growth factor A (VEGFA), a potent and widely distributed angiogenic factor, has been reported to be less frequent in MSI-H colorectal cancers than in low-frequency MSI (MSI-L) or microsatellite stable (MSS) cancers [6]. Reduced expression of prostaglandin-endoperoxide synthase 2 (PTGS2) cyclooxygenase 2 (COX2) has also been shown in sporadic and hereditary MSI-H colorectal cancers [7,8]. These findings are interesting, because PTGS2 has been shown to regulate angiogenesis by promoting the production of VEGFA and fibroblast growth factor 2 (FGF2) [9].

Molecular and clinicopathological distinctions between cancers with and those without MSI-H are also evident in gastric cancers [10,11]. Therefore, gastric cancers with and those without MSI-H are thought to represent distinctive pathways of carcinogenesis [10,11]. VEGFA expression, determined by immunohistochemistry, in gastric cancer cells has been reported to be correlated with vascular invasion and lymph node and liver metastasis [12]. We and others have reported that p53 mutations are less frequent in MSI-H gastric cancers than in MSI-L or MSS cancers [11,13]. We have also shown that overexpression of PTGS2 is less frequent in MSI-H gastric cancers [14]. A significant association between p53 mutation and VEGFA expression has been reported in colon cancer [15,16]. p53 has also been shown to repress FGF2 mRNA translation by a direct mechanism involving its nucleic acid unwinding-annealing activity [17]. These findings led us to hypothesize that expression of VEGFA and/or FGF2 is less frequent in gastric cancers with MSI-H. In contrast to colorectal cancers, however, little is known about the relationships of expression of VEGFA and FGF2 with MSI status, p53 mutations, and PTGS2 expression in gastric cancers.

The local balance between various molecules that induce and inhibit neovascularization affects angiogenesis. Either increased production of activators or decreased production of inhibitors increases angiogenesis. In this regard, it is of interest that a frequent inactivation of the thrombospondin 1 (THBS1) gene by methylation has been demonstrated in colon cancers with MSI-H [18,19]. THBS1 is a multifunctional protein implicated in cancer cell adhesion, migration, invasion, inhibition of angiogenesis, and activation of latent transforming growth factor β. THBS1 is an angiogenesis inhibitor with tumor suppressor properties [20,21]. An inhibitory effect of THBS1 on angiogenesis, which could potentially inhibit both tumorigenesis and metastasis has been shown in a number of studies [22]. Interestingly, THBS1 has also been identified as a direct transcriptional target of p53 [23,24]. One mechanism by which wild-type p53 may exert its tumor suppressor function is thought to be the THBS1-dependent inhibition of tumor angiogenesis. THBS1 was originally described in the CpG island methylator phenotype (CIMP) panel along with MINTs [19]. Therefore, the wild-type p53 and/or CIMP in MSI-H gastric cancers may create an additional selective pressure to suppress THBS1 expression [25]. However, a correlation of THBS1 methylation and MSI status is not known in gastric cancer, although THBS1 methylation has been reported in a small number of gastric cancer tissue samples analyzed [26–28]. In addition, several factors such as Wilms tumor 1 (WT1), has also been reported to be involved in the regulation of THBS1 expression in tumor [29].

An antiangiogenic factor, brain-specific angiogenesis inhibitor 1 (BAI1), is also a p53 target molecule [30]. BAI1 is a specific inhibitor of endothelial cell migration and contains several discrete functional domains that can inhibit experimental angiogenesis. BAI1 contains 5 THBS type I repeats and reportedly inhibits in vivo neovascularization induced by FGF2 in the rat cornea [30]. Expression of BAI1 was absent or significantly reduced in 8 of 9 glioblastoma cell lines, suggesting that BAI1 plays a significant role in angiogenesis inhibition, as a mediator of p53 [30].

Since molecular therapeutics targeting tumor angiogenesis are already being used in clinical settings and trials [31,32], it seems important to clarify if expression of genes involved in angiogenesis is differentially regulated between gastric cancers with and those without MSI-H. In this study, we systematically analyzed the expression of VEGFA, FGF2, THBS1, WT1, and BAI1, and we correlated the results with microvessel count (MVC), MSI status, p53 mutations, and PTGS2 expression in gastric cancers.