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ARTICLE

High Production of SPARC/osteonectin/BM-40 in Mouse Metastatic B16 Melanoma Cell Lines*

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Production of SPARC/osteonectin/BM-40 was determined in mouse B16 melanoma clones BL6 and F10 (high metastatic) and F1 (low metastatic). SPARC was produced greater amount in BL6 and F10 than in F1 cells, showing a good agreement with their metastatic potentials. Moreover, SPARC production was not influenced by culture pH, even in the acidic conditions (≈pH 5.9). Although tumor tissues show often low pH due to excessive amount of acidic metabolites such as lactate, most studies have been done in neutral pH. High SPARC production in the acidic medium, therefore, is thought to be an important potential for tumor invasive behaviour. (Pathology Oncology Research Vol 6, No 1, 24–26, 2000)

Keywords: SPARC, osteonectin, BM-40, melanoma, metastasis

Introduction

SPARC (secreted protein, acidic and rich in cysteine)/osteonectin/BM-40 is a secreted Ca^{2+}-binding glycoprotein of 43 kDa.[1-3] SPARC expression has been shown in some specific tumors such as breast,[4] colorectal,[5] esophageal[6] cancers, melanoma,[7,8] astrocytoma,[9] meningioma,[10] and hepatocellular carcinoma.[11] Ledda et al[12] reported that SPARC antisense RNA abrogates the tumorigenicity of human melanoma cells. Some reports have shown the relationship between SPARC and matrix metalloproteinase (MMP) secretions. For example, SPARC is co-expressed with stromelysin-3 in colorectal cancer,[5] and induces production of collagenase, stromelysin and gelatinase B,[13,14] and mediates pro-gelatinase A activation via membrane type 1-MMP (MT1-MMP).[15] In addition, it stimulates migration of human renal cell carcinoma[16] and prostatic cancer.[17] Thus, SPARC is thought to play a role of tumorigenesis and tumor progression.

We have previously reported that the expression of gelatinase B by high metastatic mouse B16 melanoma clones (F10 and BL6) is stimulated by acidic culture conditions.[18,19] As Martinez-Zaguilán et al[20] pointed out, despite the acidity of tumors, most *in vitro* assays of tumor cell function are routinely performed at neutral-to-alkaline medium pH. We therefore evaluated SPARC production in B16 melanoma clones cultured with neutral or acidic medium, and compared with their metastatic potentials in this study.

Materials and Methods

Cell culture

B16 variants (high metastatic BL6 and F10 and low metastatic F1) were used in this study. They were maintained in DME/F12 (Gibco/BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Sera-Lab, Sussex, UK).

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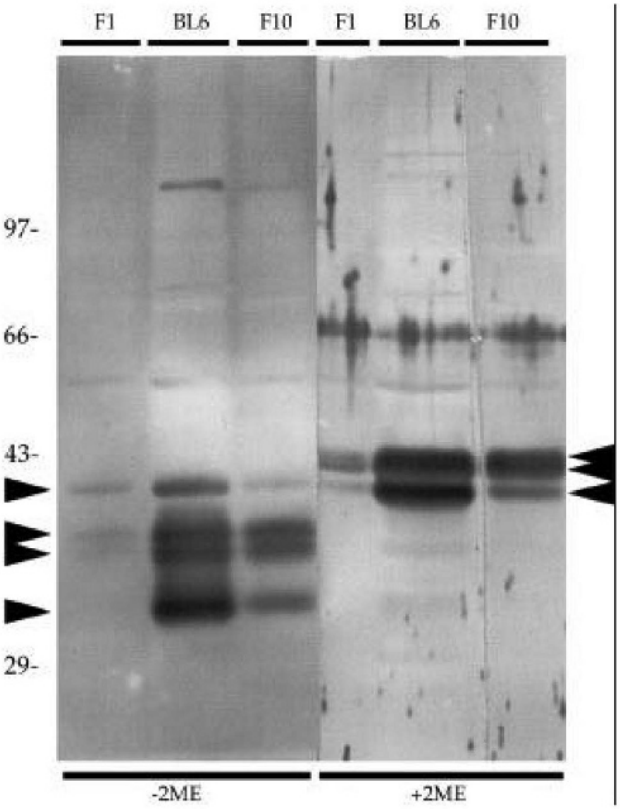

Figure 1. *SPARC production by mouse B16 melanoma cells in neutral culture conditions (pH 7.3). Samples (10 µg protein) were separated by 10% SDS-PAGE under non reducing (–2ME) or reducing (+2ME) conditions. Western blotting was performed using SPARC polyclonal antibody. Arrow heads, SPARC.*

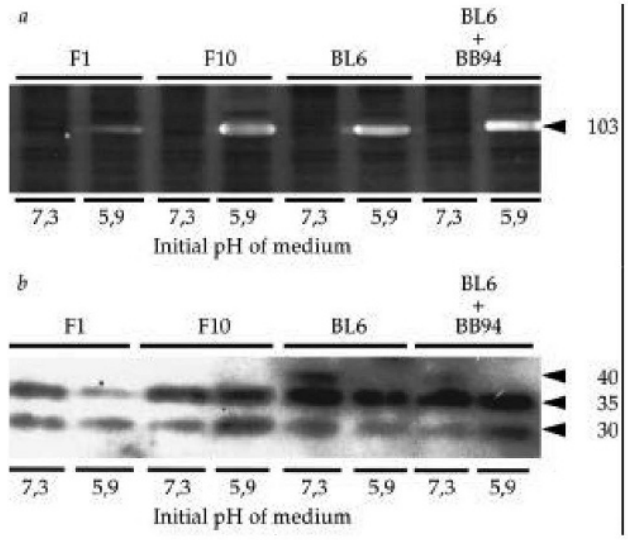

Figure 2. *Production of gelatinase B and SPARC by mouse B16 melanoma cell lines in the acidic culture conditions (pH 5.9). Cells were maintained in serum-free DME/F12 with pH 5.9 and 7.3 for 3 days. Concentrated CM (10 µg protein) was subjected to zymography (a) and Western blotting (b). Arrow, gelatinase B activity; arrow heads, SPARC.*

Preparation of conditioned medium (CM)

Sub-confluent cultures were maintained in serum-free DME/F12 with pH 5.9 or 7.3 for 3 days as described previously.[18] In the case of BL6 cells, they were cultured in the serum-free media for 3 days in the presence or absence of 10^{-6} M BB-94 (British Biotech, Oxford, UK). CM was collected, concentrated by precipitation with ammonium sulfate at 80% saturation.

Zymography

Gelatinase B activity was measured by gelatin-zymography as reported previously.[18] The proteins (10 µg) in CM were electrophoresed on 10% polyacrylamide gels containing 0.1% gelatin as the substrate. SDS was removed from the gels by washing with 2.5% Triton X-100. Enzyme reaction was carried out by incubating in the presence of 10 mM $CaCl_2$ at 37°C for 20 h. The resultant gels were stained with Coomassie Brilliant Blue R-250.

Western blotting

Samples (10 µg protein) were separated by 10% SDS-PAGE under non reducing or reducing conditions and transferred onto Immobilon-P membrane (Millipore, Bedford, MA, USA). SPARC polyclonal antibodies (kind gifts from Dr. L. Fisher and Prof. E.H. Sage) and peroxidase-conjugated anti-rabbit IgG (Dako, Copenhagen, Denmark) were used as first and second antibody, respectively. Peroxidase was revealed by the enhanced chemiluminescence assay. (Amersham, Buckinghamshire, UK).

Results and Discussion

SPARC proteins, secreted in neutral pH (7.3), were detected by Western blot as 3 bands (43-, 42-, 40-kDa) in the reducing conditions and 4 bands (40-, 36-, 35-, 30-kDa) in the non reducing conditions, respectively ***(Figure 1).*** SPARC proteins were detected highly in BL6 and F10, but faint in F1 cells. As previously reported elsewhere,[18] the secretion of gelatinase B by F10 and BL6 cells was considerably stimulated by acidic culture medium (pH 5.9) ***(Figure 2a).*** Their SPARC production remained identical with an identical pattern at pH 5.9 and 7.3 ***(Figure 2b).***

It has been reported that SPARC was degraded by several MMPs including gelatinase B.[21] Our results, however, did not show any significant degradation of SPARC at acidic pH, that would have been expected from gelatinase B overexpression. Moreover, the addition of BB-94, a synthetic MMP inhibitor, to the cultures did not affect SPARC electrophoretic mobility, suggesting that the several electrophoretic mobilities of SPARC bands could not be the consequence of proteolysis by MMPs ***(Figure 2b).*** The fact

that activation of gelatinase B was not significantly caused by acidic culture conditions also might deny participated in MMPs for protein ladder of SPARC. The different bands of SPARC may correspond to different glycosylated forms rather than to different degradation products. As previous reports mentioned that SPARC induced MMP production or activation, we expected that increased gelatinase B production in acidic medium pH is caused by over expression of SPARC as an autocrine manner. However, the culture medium that was conditioned at pH 5.9 and then neutralized, and that is considered to be high SPARC content as shown in figure 2, did not change gelatinase B production in F10 cells,[19] suggesting that production of SPARC and gelatinase B is regulated independently.

Rempel et al[10] have found that invasive meningiomas produced excessive amount of SPARC independently of grade, and that the increased SPARC expression promoted glioblastoma *in vitro* invasion. SPARC also induced cell motility of renal cell carcinoma associated with type IV collagen.[16] In addition, SPARC production was well correlated with metastatic ability of mouse B16 melanoma cells in this study. Taken together, SPARC action might be a most effective in tumor intravasation or extravasation in the tumor metastasis process. The high expression of SPARC and gelatinase B particularly at acidic pH conditions therefore may independently support the metastatic behaviour of melanoma cells.

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