Motility Inhibition and Apoptosis Are Induced by Metastasis-suppressing Gene Product CD82 and Its Analogue CD9, with Concurrent Glycosylation

Masaya Ono, Kazuko Handa, Donald A. Withers, and Sen-itiroh Hakomori

Pacific Northwest Research Institute, Seattle, Washington 98122, and Departments of Pathobiology and Microbiology, University of Washington, Seattle, Washington 98195

Abstract

Metastasis-suppressing gene product CD82 and its analogue CD9 are considered to suppress the malignancy of various human cancers, although the rationale for this effect is unknown. The present study addresses phenotypic changes in Chinese hamster ovary cell mutant line ldlD-14 mutant medium. These changes reflect not only the effect of CD82 or CD9 expression, but also the effect of endogenous GM3 synthesis. The effect of glycosylation on cellular function was determined on day 3 for cell migration and on day 10–11 for apoptosis.

Preparation of cDNA Encoding CD82 and CD9, and Its Transfection into ldlD-14 Cells. cDNAs for CD82 and CD9 were generated by reverse transcription-PCR using human placental RNA (Clontech, Palo Alto, CA) and Superscript II reverse transcriptase (Life Technologies, Inc., Gaithersburg, MD). PCR amplification with Elongase (Life Technologies, Inc.) was performed with 5′ primer AGAGGAAATTCCATGGCCGTCAAAAGGAGG and 3′ primer GAGGGGATCCTTCCTGTCAGGGATGTAAG for CD82 and with 5′ primer CGCGGAAATTTGGAGCTGCTAGGGCCGTCAAAAGGAGG and 3′ primer GCCGGCTCAGTCAACTTTCCGAGGACCTGC for CD9. The resulting PCR products were cloned into pCDNA3 (Invitrogen, Carlsbad, CA) and sequenced to confirm identity by comparison with published sequences (1, 3).

pCDNA3 containing CD9 and CD82 was transfected into ldlD-14 cells using LipofectAMINE (Life Technologies, Inc.), transfectants were isolated by growing them in G418 (Life Technologies, Inc.)-containing medium, and clones were selected by expression of the respective antigens probed by the mAb, as described previously (9).

Haptotactic and Phagokinetic Motility Assay. Transwell assay using a 6.5-mm transwell assembly (8-μm pore size; Costar, Cambridge, MA; Refs. 10 and 11) and the gold sol phagokinetic assay (12) were performed as described previously. Some modifications of the procedures are explained in the legend of Fig. 2.

Determination of GM3 and Other GSLs. Packed cells (106 cells harvested for one run) were extracted in 2 ml of chloroform-methanol (2:1 by volume) by sonication for 5 min and centrifuged, and the pellet was extracted two more times with the same solvent. The GSL fraction from the combined extract was separated using an acetylation procedure (13).

Determination of CD9 and CD82 with Glycosylation Status. Cells were solubilized in lysis buffer, subjected to SDS-PAGE, electrophoretically transferred to Immobilon-P (Millipore, Bedford, MA), and subjected to Western blotting with a chemiluminescence system (Pierce, Rockford, IL; Ref. 11). Protein content was determined with a BCA kit (Pierce). Antihuman CD9 mouse mAb M-L13 was from PharMingen (San Diego, CA). Antihuman CD82 mouse mAb B-L2 was from Serotec (Raleigh, NC).

Determination of Apoptosis. Morphological changes characteristic of apoptosis (14), i.e., nuclear compaction, cytoplasmic condensation, and disintegration into dense particles, were examined. DNA fragmentation was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (15) using the in situ cell death detection kit (Boehringer Mannheim, Indianapolis, IN). Translocation of phosphatidylserine associated with apoptosis (16) was determined by annexin-V-fluos staining kit (Boehringer Mannheim).

Results and Discussion

CD82- and CD9-expressing ldlD-14 Cells. ldlD-14 clones expressing CD82 (ldlD/CD82; clones 515 and 1209) and clones expressing CD9 (ldlD/CD9; clones 24, 10, and 47) were established (Fig. 1A). SDS-PAGE and Western blotting indicate that: (a)
the molecular mass (23 kDa) of CD9 in ldlD/CD9 cells was unchanged, regardless of the addition of Gal (Fig. 1 B, a); (b) CD82 had a much higher molecular mass and a broader band (45–65 kDa) in ldlD/CD82 cells grown in the presence of Gal or Gal plus GalNAc (Fig. 1 B, b); and (c) endogenous GM3 synthesis occurred for both CD9- and CD82-expressing cells grown in the presence of Gal or Gal plus GalNAc (Fig. 1 C).

Only CD9- or CD82-expressing Cells with Concurrent GM3 Synthesis and Complete N-Glycosylation Display Inhibition of Haptotactic and Phagokinetic Motility. Haptotactic motility of ldlD/CD9 clones 24 and 28 (Fig. 2 A) and ldlD/CD82 clones 515 and 1209 (Fig. 2 B) as well as the phagokinetic motility of ldlD/CD9 clones 24 and 47 (Fig. 2 D) and ldlD/CD82 clones 515 and 1209 (Fig. 2 E) were all reduced in cells grown in the presence of Gal or Gal plus GalNAc, but not those grown in the presence of GalNAc alone. Parental ldlD cells, which do not express CD9 or CD82, showed no change of motility on the addition of Gal or GalNAc (Figs. 2 C and F).

Apoptosis Was Induced Only in CD9 and CD82-expressing Cells, with Concurrent GM3 Synthesis and Complete N-Glycosylation. Massive cell death (apoptosis) occurred after 11 days in ldlD/CD9 (Fig. 3 A, first column) or ldlD/CD82 (second column) culture in ITS added with Gal (Fig. 3 A, row b) or with Gal plus GalNAc (row c). Cell death did not occur in ITS medium without Gal addition (row a) or for parental ldlD14 cells, regardless of Gal addition (third column). The effect of CD9 and CD82 with concurrent glycosylation (Gal addition) on the induction of apoptosis was confirmed by flow cytometry after nick end labeling, i.e. 3' hydroxyl end detected on day 11 in cells cultured on a Matrigel-coated plate. Clear labeling was observed in ldlD/CD9 and ldlD/CD82 in Gal-supplemented medium (Fig. 3 B, row b) and in Gal plus GalNAc-supplemented medium (row c). Labeling was minimal in ITS medium without Gal addition (row a) or for parental ldlD14 cells, regardless of Gal addition (third column).

Changes in the number of live cells/mm² are shown in Fig. 4. The number of ldlD/CD9 and ldlD/CD82 cells, but not of parental ldlD14
cells decreased significantly upon culture in Gal or Gal plus GalNAc-supplemented medium on Matrigel-coated plates (Fig. 4A) or non-coated plates (Fig. 4B).

Dead cells in the above experiments showed a typical apoptotic morphology (nuclear compaction, cytoplasmic condensation, and disintegration into dense particles).

The Malignancy-suppressing Effect of CD82 and CD9 Is Due to Motility Inhibition and Apoptosis and Is Promoted by Concurrent GM3 Synthesis and Complete N-Glycosylation. CD82 (KAI-1) was originally assigned as a metastasis-suppressing gene product, and its expression was shown to be down-regulated in a few types of human cancer (1, 2, 5). Its analogue, CD9, is also down-regulated in metastatic tumors (3, 4). Expression of both CD82 and CD9 was correlated with the patient survival rate (2, 4, 5). However, the cell biological mechanism for these effects of CD82 and CD9 is unknown.

Results of the present study indicate that expression of both CD82 and CD9 inhibits haptotactic and phagokinetic cell motility and induces cell death with features typical of apoptosis, and that this process is promoted by concurrent GM3 synthesis and complete N-glycosylation. Because N-glycosylation in CD9 is minimal, endogenous GM3 synthesis has the major effect on CD9. On the other hand, there is a high level of N-glycosylation in CD82, as indicated by the great increase in molecular mass and heterogeneity in ldlD/CD82 cells grown in Gal-supplemented medium. Inhibition of cell motility and induction of apoptosis in ldlD/CD82 cells are ascribable to enhanced N-glycosylation. The effect of endogenous GM3 synthesis on CD82 must be similar to the effect on CD9, i.e. endogenous GM3 is the common factor for both CD9- and CD82-dependent inhibition of cell motility and induction of apoptosis.

Expression of CD82 or CD9 per se is an essential, but not sufficient, factor to induce cell motility inhibition and apoptosis. Completion of N-glycosylation and endogenous GM3 synthesis, as observed...
typically in ldlD cells grown in Gal-supplemented medium, are re-
quired to manifest motility inhibition as well as CD82- or CD9-
dependent induction of apoptosis, although the latter process requires
at least a 10-day latent period. What type of molecular mechanism
takes place during the latent period is yet unidentified. Little is known
about the correlation between glycosylation and apoptosis, although
Le$^a$ (17) and Le$^b$ (18) expressed in colorectal carcinoma are associ-
ated with apoptosis. Glycosylation that defines malignancy depends
largely on which molecular species is glycosylated and is susceptible
to gangliosides. Tetraspan membrane proteins that suppress tumor

Fig. 3. CD9- and CD82-dependent apoptosis promoted by glycosylation. A, morphology change of ldlD cells at day 11 on a Matrigel-coated plate. Three cell lines (as indicated
at the bottom) were grown in ITS medium alone (a), ITS + Gal (b), and ITS + Gal + GalNAc (c). The number of adherent cells is dramatically decreased in Gal-containing medium
(b and c) for CD9- and CD82-expressing cells but is minimally changed for parental ldlD14 cells. Dead cells are characterized by round shape, nuclear compaction, cytoplasmic
condensation, and disintegration into dense particles. B, flow cytometric patterns showing nick end labeling of ldlD/CD9, ldlD/CD82, and parental ldlD cells. Cells were cultured on
Matrigel-coated plates in ITS medium (top row), ITS + Gal (second row), or ITS + Gal + GalNAc (third row) and stained on day 11. Note that the high fluorescence peak representing
apoptotic cells is clearly observed only in CD9- or CD82-transfected cells in the presence of Gal. This trend is much weaker in parental cells without CD9 or CD82.
malignancy are undoubtedly target molecules of glycosylation, controlling motility and apoptosis.

Acknowledgments

We thank Dr. Monty Krieger for the donation of ldlD-14 cells, Wendy Smith and Jon McBride for technical assistance, and Dr. Stephen Anderson for scientific editing and preparation of the manuscript.

References


Motility Inhibition and Apoptosis Are Induced by Metastasis-suppressing Gene Product CD82 and Its Analogue CD9, with Concurrent Glycosylation

Masaya Ono, Kazuko Handa, Donald A. Withers, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/10/2335

Cited articles
This article cites 18 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/10/2335.full.html#ref-list-1

Citing articles
This article has been cited by 41 HighWire-hosted articles. Access the articles at:
/content/59/10/2335.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.