Brazilian Journal of Medical and Biological Research (1999) 32: 891-895
ISSN 0100-879X

Oncogene-mediated downregulation of *RECK*, a novel transformation suppressor gene

R.M. Sasahara[1], C. Takahashi[2], M.C. Sogayar[1] and M. Noda[2]

[1]Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brasil
[2]Department of Molecular Oncology, Kyoto University Graduate School of Medicine, Yoshida-Konoe-cho, Sakyo-ku, Kyoto, Japan

Abstract

The *RECK* gene was initially isolated as a transformation suppressor gene encoding a novel membrane-anchored glycoprotein and later found to suppress tumor invasion and metastasis by regulating matrix metalloproteinase-9. Its expression is ubiquitous in normal tissues, but undetectable in many tumor cell lines and in fibroblastic lines transformed by various oncogenes. The *RECK* gene promoter has been cloned and characterized. One of the elements responsible for the oncogene-mediated downregulation of mouse *RECK* gene is the Sp1 site, where the Sp1 and Sp3 factors bind. Sp1 transcription factor family is involved in the basal level of promoter activity of many genes, as well as in dynamic regulation of gene expression; in a majority of cases as a positive regulator, or, as exemplified by the oncogene-mediated suppression of *RECK* gene expression, as a negative transcription regulator. The molecular mechanisms of the downregulation of mouse *RECK* gene and other tumor suppressor genes are just beginning to be uncovered. Understanding the regulation of these genes may help to develop strategies to restore their expression in tumor cells and, hence, suppress the cells' malignant behavior.

Key words
- *RECK* transformation suppressor gene
- Reversion
- Transcriptional regulation
- Sp1 family
- Tumor suppressor genes

Correspondence
R.M. Sasahara
Instituto de Química, USP
Caixa Postal 26077
05599-970 São Paulo, SP
Brasil
Fax: +55-11-818-3820
E-mail: sasahar@quim.iq.usp.br

Presented at the I International Symposium on "Signal Transduction and Gene Expression in Cell Proliferation and Differentiation", São Paulo, SP, Brasil, August 31-September 2, 1998.

Research supported by FAPESP, CNPq, PADCT, ICGEB, FBB, PRP-USP, Japanese Foundation for Cancer Research, Japanese Ministry of Education, Science, Sports and Culture, Science and Technology Agency of Japanese Government, Amgen, Inc. R.M. Sasahara was the recipient of a Japanese Government fellowship and of a FAPESP pre-doctoral fellowship.

Received November 27, 1998
Accepted January 11, 1999

Introduction

Ras genes are frequently mutated in many types of human tumors (1). Oncogenically mutated forms of *ras* genes induce dramatic morphological alterations and anchorage-independent growth in various cells including rodent fibroblasts (2). Because of their importance in human tumors and the ease in studying the phenotypic changes induced by them *in vitro*, *ras* genes have been frequently used as a target for the study of reversion (3).

As an approach toward understanding the mechanism of cell transformation induced by activated *ras* genes, morphologically nontransformed ("flat") revertants (4,5) from a transformed subline of NIH/3T3 (the "DT" cell line) containing Kirsten murine sarcoma virus (a transforming virus carrying the *v-Ki-ras* gene) have been isolated following transfection of a human fibroblast complementary DNA (cDNA) expression library. The *Krev-1* gene (6), also known as *rap1A*, which encodes a Ras-related protein

containing a region identical to the effector domain of Ras, was isolated in a previous study (7) using a plasmid-based human fibroblasts cDNA expression library. Using a similar approach, Cutler et al. (8) isolated another transformation suppressor gene, *rsp-1*, encoding a leucine-rich repeat protein. A similar screening of a human fibroblast cDNA expression library constructed with a new phagemid shuttle vector resulted in the isolation of two cDNA clones exhibiting significant biological activities. One of these, clone CT124, was found to encode a truncated form of the MSX-2 homeobox protein which induces flat reversion through a dominant negative mechanism on the endogenous MSX-2 protein (9). The other reversion-inducing clone corresponds to the *RECK* gene, which encodes a novel membrane-anchored glycoprotein of about 110 kDa with multiple epidermal growth factor (EGF)-like repeats and serine protease inhibitor-like domains (10).

The *RECK* gene is widely expressed in normal tissues, while in several tumor cell lines and oncogene-transformed fibroblasts, it is downregulated (10). These results suggest that the *RECK* gene is a common negative target for oncogenic signals, linking these signals to malignant conversion. Restoration of RECK expression in malignant cells resulted in suppression of invasive activity with a concomitant decrease in the secretion of matrix metalloproteinase-9 (MMP-9), a key enzyme involved in tumor invasion and metastasis (10). Biochemical assays showed that purified RECK protein binds to and inhibits the proteolytic activity of MMP-9 (10). These findings are consistent with a model in which the release of MMP-9 is somehow gated by the membrane-anchored human RECK protein on the plasma membrane. When an oncogenic signal is turned on, the *RECK* gene is downregulated, resulting in increased secretion of MMP-9, which contributes to morphological transformation as well as to the invasive behavior of the cells (10). Therefore, downregulation of the *RECK* gene by these signals has been analyzed in order to gain important insights into the mechanism of oncogenic signal transduction and malignant conversion (Sasahara RM, Takahashi C and Noda M, unpublished results).

Regulation of mouse *RECK* gene expression by Sp1 transcription factor family members

Analysis of the mouse *RECK* gene promoter has indicated that the oncogene-induced downregulation of this gene is at least partially mediated by the Sp1/Sp3 binding sites immediately downstream of the transcription initiation site (Sasahara RM, Takahashi C and Noda M, unpublished results).

The Sp1 transcription factor activates transcription by associating with one of the TATA-binding protein (TBP) co-activators in the TFIID complex. Interaction between glutamine-rich activation domains of Sp1 and the TBP-associated factor dTAF is an important component of the Sp1 transactivating activity (11). Since the TFIID complex and TBP-associated cofactors also play an essential role in the activation of TATA-less promoters, the mechanism by which Sp1 activates these two types of promoters may be based on common features (12). In the majority of the promoters containing Sp1-binding elements, Sp1 appears to provide a basal level of transcription, but, when acting in conjunction with other transcriptional activators or regulatory proteins, Sp1 can also participate in the dynamic regulation of gene expression. Sp1 activates transcription by cooperative interaction either with itself (13) or with other transcriptional factors, such as the E2 protein bound to the bovine papillomavirus enhancer (14), Tat protein bound to the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (15), NF-kBp65 bound to the HIV-1

enhancer (16,17), and retinoblastoma protein (pRb) bound to the retinoblastoma control element in c-fos, c-myc, and transforming growth factor (TGF) beta1 genes (18). GATA-1, the major erythroid transcription factor, physically interacts with Sp1 and activates transcription in a synergistic manner (19). Sp1 and the immediate-early gene product Egr-1 physically and functionally cooperate to mediate maximal interleukin-2 receptor ß-chain promoter activity (20). Sp1 plays a critical role in cytokine-stimulated expression of the vascular cell adhesion molecule gene (21). The myeloid-cell-specific expression of the CD11c gene was shown to be due to the binding of Sp1 to the CD11c promoter region and its interaction with Ap1 (22). Sp1 has also been shown to be involved in the Ras/Raf pathway. The Raf-mediated signaling pathway leads to alterations in Sp1 activity, resulting in higher levels of transcription of two growth-responsive genes, namely, rep-3b and mdr1 (23). Sp1-binding sequences were found to be critical for the Ha-ras effect of activating transcription of the human 12-lipoxygenase gene (24). However, in the examples cited above, Sp1 acts as a positive regulatory element, in contrast to the oncogene-responsive Sp1 site in the mouse RECK promoter.

The existence of a family of Sp transcription factors (25,26) suggests that gene regulation by Sp1 is more complex than previously assumed. Sp3, a member of this family, was initially found to suppress Sp1-mediated transcripton activation by competitively binding to Sp1 consensus elements (16,27) or by functioning as a repressor by protein-protein interaction (28). Sp3 can also stimulate transcription, as reported for the PDGF-B promoter (29) and for promoters containing the pRb control elements, by functional interaction with pRb (30). Finally, Sp3 was reported to be a dual-function regulator whose activity is dependent upon both the promoter and the cellular context (28). Therefore, an obvious possibility, in the case of downregulation of mouse *RECK* gene expression through the Sp1 site, would be that Sp3 is induced or somehow activated in oncogene-transformed cells, thus occupying and blocking the Sp1 site. However, this hypothesis has not been confirmed (Sasahara RM, Takahashi C and Noda M, unpublished results). Interestingly, it was recently reported that Sp1 plays a critical role in Erb-B2 and v-ras-mediated downregulation of the alpha2-integrin gene in human mammary epithelial cells (31). In that case, however, in contrast to the oncogene-mediated downregulation of mouse *RECK* gene (Sasahara RM, Takahashi C and Noda M, unpublished results), a slight reduction in the binding of Sp1 to the critical Sp1 site was observed (31). Thus, multiple mechanisms may exist in oncogene-mediated transcriptional suppression through Sp1 sites. Sp1 can be regulated via post-translational modifications of its transactivation domain, such as O-linked glycosylation (32) and phosphorylation (33). One possibility is that oncogene products suppress mouse *RECK* gene expression by affecting such post-translational modifications. Another possibility is that oncogene signaling affects the interaction between Sp1/Sp3 and their regulatory protein(s). Notably, a 74,000-Mr protein that binds the transactivation domain of Sp1 and substantially inhibits Sp1-mediated transactivation *in vivo* was identified (34). The mechanism of oncogene-mediated downregulation of the *RECK* gene through the Sp1 site is still to be elucidated, but the identification of the Sp1 site as the responsive element provided evidence that this binding site may serve as a negative regulatory element in certain promoter and/or cellular contexts (Sasahara RM, Takahashi C and Noda M, unpublished results).

Transcriptional regulation of tumor suppressor genes

Genes that are downregulated in the pro-

cess of carcinogenesis are important candidates as tumor suppressors. Expression of E-cadherin, a potential invasion-suppressor gene, is downregulated in human carcinomas by transcriptional inactivation through CpG hypermethylation (35,36). The expression of tumor suppressor genes such as p16 (37), pRb (38), and p53 (39) is also suppressed by methylation of CpG islands in the respective 5'-control regions. Expression of maspin, a serine protease inhibitor with tumor/metastasis-suppressing activity in the mammary gland, is lost during tumor progression as a result of decreased transactivation through the Ets and Ap1 sites (40). Alpha2-integrin also plays important roles in the malignant behavior of tumor cells and is downregulated by oncogene products, as mentioned above (31). In addition to *RECK*, another protease inhibitor gene that is negatively regulated by the *ras* oncogene product has recently been found (Izumi H and Noda M, unpublished results). It will be interesting to investigate whether a common mechanism underlies the downregulating effect of these genes.

Future perspectives

Further characterization of the oncogene-responsive elements present in the mouse *RECK* promoter should contribute to defining the molecular interplay among multiple cis-acting elements and trans-acting factors in the regulation of mouse *RECK* gene expression. Other regulatory events, such as DNA methylation of the endogenous mouse *RECK* promoter and nucleosome phasing at specific activation sites, may confer a higher level of specificity and complexity in the regulation of mouse *RECK* expression *in vivo*. A better understanding of the mechanisms of regulation of *RECK* gene transcription should allow further elucidation of *RECK* gene downregulation in the malignant conversion of tumor cells.

To understand how different oncogene products modulate transformation-suppressor genes may also help to develop strategies to restore the expression of the latter in tumor cells and, hence, to suppress the malignant phenotype.

References

1. Bos JL (1989). Ras oncogenes in human cancer: a review. *Cancer Research*, 49: 4682-4689.
2. Barbacid M (1987). Ras genes. *Annual Review of Biochemistry*, 56: 779-827.
3. Noda M (1993). Mechanisms of reversion. *FASEB Journal*, 7: 834-840.
4. Noda M, Selinger Z, Scolnich EM & Bassin RH (1983). Flat revertants isolated from Kirsten sarcoma virus-transformed cells are resistant to the action of specific oncogenes. *Proceedings of the National Academy of Sciences, USA*, 80: 5602-5606.
5. Bassin RH & Noda M (1987). Oncogene inhibition by cellular genes. *Advances in Viral Oncology*, 6: 103-127.
6. Kitayama H, Sugimoto Y, Matsuzaki T, Ikawa Y & Noda M (1989). A ras-related gene with transformation suppressor activity. *Cell*, 56: 77-84.
7. Noda M, Kitayama H, Sugimoto Y, Okayama H, Bassin RH & Ikawa Y (1989). Detection of genes with a potential for suppressing the transformed phenotype associated with activated ras genes. *Proceedings of the National Academy of Sciences, USA*, 86: 162-166.
8. Cutler ML, Bassin RH, Zanoni L & Talbot N (1992). Isolation of rsp-1, a novel cDNA capable of suppressing v-Ras transformation. *Molecular and Cellular Biology*, 12: 3750-3756.
9. Takahashi C, Akiyama N, Matsuzaki T, Takai S, Kitayama H & Noda M (1996). Characterization of a human MSX-2 cDNA and its fragment isolated as a transformation suppressor gene against v-Ki-ras oncogene. *Oncogene*, 12: 2137-2146.
10. Takahashi C, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, Ikawa Y, Ratzkin BJ, Arakawa T & Noda M (1998). Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. *Proceedings of the National Academy of Sciences, USA,* 95: 13221-13226.
11. Gill G, Pascal E, Tseng ZH & Tjian R (1994). A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAFII110 component of the *Drosophila* TFIID complex and mediates transcriptional activation. *Proceedings of the National Academy of Sciences, USA,* 91: 192-196.
12. Pugh BF & Tjian R (1991). Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes and Development,* 5: 1935-1945.
13. Su W, Jackson S, Tjian R & Echols H (1991). DNA looping between sites for transcriptional activation: self-association of DNA-bound Sp1. *Genes and Development*, 5: 820-826.
14. Li R, Knight JD, Jackson SP, Tjian R & Botchan MR (1991). Direct interaction be-

tween Sp1 and the BPV enhancer E2 protein mediates synergistic activation of transcription. *Cell*, 65: 493-505.
15. Huang L & Jeang K (1993). Increased spacing between Sp1 and TATAA renders human immunodeficiency virus type 1 replication defective: implication for Tat function. *Journal of Virology*, 67: 6937-6944.
16. Majello B, De Luca P, Hagen G, Suske G & Lania L (1994). Different members of the Sp1 multigene family exert opposite transcriptional regulation of the long terminal repeat of HIV-1. *Nucleic Acids Research*, 22: 4914-4921.
17. Perkins ND, Agranoff AB, Pascal E & Nabel GJ (1994). An interaction between the DNA-binding domains of Rel A (p65) and Sp1 mediates human immunodeficiency virus gene activation. *Molecular and Cellular Biology*, 14: 6570-6583.
18. Udvadia AJ, Rogers KT, Higgins PD, Murata Y, Martins KH, Humphrey PA & Horowitz JM (1993). Sp1-binds promoter elements regulated by the RB protein and Sp-1 mediated transcription is stimulated by RB coexpression. *Proceedings of the National Academy of Sciences, USA*, 90: 3265-3269.
19. Merika M & Orkins SH (1995). Functional synergy and physical interactions of the erythroid transcription factor GATA-1 with the Kruppel family proteins Sp1 and EKLF. *Molecular and Cellular Biology*, 15: 2437-2447.
20. Lin J & Leonard WJ (1997). The immediate-early gene product Egr-1 regulates the human interleukin-2 receptor beta-chain promoter through noncanonical Egr and Sp1 binding sites. *Molecular and Cellular Biology*, 17: 3714-3722.
21. Neish AS, Read MA, Thanos D, Pine R, Maniats T & Collins T (1995). Endothelial interferon regulatory factor 1 cooperates with NF-kB as a transcriptional activator of vascular cell adhesion molecule 1. *Molecular and Cellular Biology*, 15: 2558-2569.
22. Noti JD, Reinemann RC & Petrus MN (1996). Sp1 binds two sites in the CD11c promoter *in vivo* specifically in myeloid cells and cooperates with Ap1 to activate transcription. *Molecular and Cellular Biology*, 16: 2940-2950.
23. Miltenberger RJ, Farnham PJ, Smith DE, Stommel JM & Cornwell MM (1995). V-Raf activates transcription of growth-responsive promoters via GC-rich sequences that bind the transcription factor Sp1. *Cell Growth and Differentiation*, 6: 549-556.
24. Chen B, Liu Y, Yamamoto S & Chang W (1997). Overexpression of Ha-ras enhances the transcription of human arachidonate 12-lipoxygenase promoter in A431 cells. *Biochimica et Biophysica Acta*, 1344: 270-277.
25. Hagen G, Muller S, Beato M & Suske G (1992). Cloning by recognition site screening of two novel GT box binding proteins: a family of Sp1 related genes. *Nucleic Acids Research*, 20: 5519-5525.
26. Kingsley C & Winoto A (1992). Cloning of GT box-binding proteins: a novel Sp1 multigene family regulating T-cell receptor gene expression. *Molecular and Cellular Biology*, 12: 4251-4261.
27. Hagen G, Muller S, Beato M & Suske G (1994). Sp1-mediated transcriptional activation is repressed by Sp3. *EMBO Journal*, 13: 3843-3851.
28. Majello B, De Lucas P & Lania L (1997). Sp3 is a bifunctional transcription regulator with modular independent activator and repression domains. *Journal of Biological Chemistry*, 272: 4021-4026.
29. Liang Y, Robinson DF, Dennig J, Suske G & Fahl WE (1996). Transcriptional regulation of the SIS/PDGF-B gene in human osteosarcoma cells by the Sp family of transcription factors. *Journal of Biological Chemistry*, 271: 11792-11797.
30. Udvadia AJ, Templeton DJ & Horowitz JM (1995). Functional interaction between the retinoblastoma (Rb) protein and Sp-family members: Superactivation by Rb requires amino acids necessary for growth suppression. *Proceedings of the National Academy of Sciences, USA*, 92: 3953-3957.
31. Ye J, Xu RH, Taylor-Papadimitriou J & Pitha PM (1996). Sp1 binding plays a critical role in Erb-B2- and v-ras-mediated downregulation of alfa2-integrin expression in human mammary epithelial cells. *Molecular and Cellular Biology*, 16: 6178-6189.
32. Jackson SP & Tjian R (1988). O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. *Cell*, 55: 125-133.
33. Jackson SP, MacDonald JJ, Lees-Miller S & Tjian R (1990). GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. *Cell*, 63: 155-165.
34. Murata Y, Kim HG, Rogers KT, Udvadia AJ & Horowitz JM (1994). Negative regulation of Sp1 trans-activation is correlated with the binding of cellular proteins to the amino terminus of the Sp1 trans-activation domain. *Journal of Biological Chemistry*, 269: 20674-20681.
35. Graff JR, Herman JG, Lapidus RG, Chopra H, Xu R, Jarrard DF, Isaacs WB, Pitha PM, Davidson NE & Baylin SB (1995). E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. *Cancer Research*, 55: 5195-5199.
36. Yoshiura K, Kanai Y, Ochiai A, Shimoyama Y, Sugimura T & Hirohashi S (1995). Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. *Proceedings of the National Academy of Sciences, USA*, 92: 7416-7419.
37. Gonzalgo ML, Hayashida T, Bender CM, Pao MM, Tsai YC, Gonzales FA, Nguyen TT & Jones PA (1998). The role of DNA methylation in expression of the p19/p16 Locus in human bladder cancer cell lines. *Cancer Research*, 58: 1245-1252.
38. Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD & Sakai T (1993). CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. *Oncogene*, 8: 1063-1067.
39. Schroeder M & Mass MJ (1997). CpG methylation inactivates the transcriptional activity of the promoter of the human p53 tumor suppressor gene. *Biochemical and Biophysical Research Communications*, 235: 403-406.
40. Zhang M, Maass N, Magit D & Sager R (1997). Transactivation through Ets and Ap1 transcription sites determines the expression of the tumor-suppressing gene maspin. *Cell Growth and Differentiation*, 8: 179-186.