Korean J Physiol Pharmacol
Vol 7: 289–293, October, 2003

Merlin Represses Ras-Induced *Cyclin D1* Transcription through the Cyclic AMP-Responsive Element

Noh-Jin Kwak[1], Hongtae Kim[1], Byung Hyune Choi[1], Young Hoon Kim[1], Hyoung Kyun Rha[1], and Kweon-Haeng Lee[1,2]

[1]Neuroscience Genome Research Center, [2]Department of Pharmacology, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea

Mutations in the *NF2* tumor suppressor gene cause neurofibromatosis type 2, an autosomal dominant inherited syndrome predisposed to the multiple tumors of the nervous system. Merlin, the *NF2* gene product was reported to block Ras-mediated cell transformation and represses Ras-induced expression of cyclin D1. However, the potential mechanism underlying the anti-Ras function of merlin on the cyclin D1 is still unclear. In this study, we investigated whether merlin decreases Ha-*ras*-induced accumulation of cyclin D1 at the transcriptional level, and demonstrated that merlin suppressed Ras-induced cyclin D1 promoter activity mediated by the cyclic AMP-responsive element (CRE) in SK-N-BE(2)C neuroblastoma cells. Furthermore, we found that merlin attenuated active Ras and forskolin-induced CRE-driven promoter activity. These results suggest that the transcriptional repression of the cyclin D1 expression by merlin may contribute to the inhibition of Ras-induced cell proliferation.

Key Words: Neurofibromatosis type 2 (NF2), Ha-*ras*, Cyclin D1, cAMP-responsive element

INTRODUCTION

NF2 (neurofibromatosis type 2) is a tumor predisposition syndrome characterized by acoustic neuromas, schwannomas, meningiomas, and ependymomas (Thomas et al, 1994). The *NF2* gene product, called schwannomin or merlin, belongs to the band 4.1 superfamily such as ERM proteins (ezrin-moesin-radixin) that links the actin cytoskeleton to the cell surface glycoproteins (Rouleau et al, 1993; Tsukita et al, 1994). Merlin and ERM proteins are able to make inter and intramolecular head-to-tail associations between the family members (Gronholm et al, 1999; Bretscher et al, 2000). Although structurally similar, merlin is unique as a tumor suppressor among the family proteins, and is known to impair cell growth, proliferation and motility (Sherman et al, 1997; Gutmann et al, 1999; Ikeda et al, 1999; Morrison et al, 2001).

The Ras family of small GTPases plays an essential role in cell proliferation and causes unlimited growth when activated (Mulcahy et al, 1985; Smith et al, 1986; Feig & Cooper, 1988; Boguski & McCormick, 1993; Mechta et al, 1997). Cyclin D1 is a prime target of the Ras signaling cascade, and is a rate-limiting component of the cell cycle regulatory holoenzyme required for G1 phase progression, cellular mitogenesis and Ras-induced transformation (Filmus et al, 1994; Liu et al, 1995; Robles et al, 1998). The cyclin D1 expression is transcriptionally induced by Ras via the cis-elements, particularly for Ets and CREB transcription factors (Tetsu & McCormick, 1999).

Previously, merlin was reported to block Ras-mediated cell transformation (Tikoo et al, 1994). We have recently shown that merlin suppresses the Ras-mediated AP-1 activity, cyclin D1 expression (Kim et al, 2002a) and Ras-ERK-mediated SRE-dependent transcriptional activity (Lim et al, 2003). Conversely, the AP-1 complex is activated, and the cyclin D1 expression increased in the *NF2* $^{-/-}$ cells, as compared with the wild-type cells (Lallemand et al, 2003; Shaw et al, 2001). Together, these studies imply that merlin blocks the oncogenic Ras signaling, which results in the inhibition of cyclin D1 accumulation.

Here, we investigated whether merlin decreases cyclin D1 expression by the activated Ras at the transcriptional level, and showed that merlin inhibits the Ras-mediated cyclin D1 transcription through the cyclic AMP-responsive element (CRE) in the promoter.

METHODS

Plasmid

The cyclin D1 promoter constructs pXP2 D1-96, D1-96m and D1-29 are kind gifts of Dr. Gerard Redeuilh (INSERM, France); pcDNA-NF2 was kindly provided by Dr. David Gutmann (Washington University, USA); The Bcl-2 promoter construct was kindly provided by Dr. Kim H (Catholic

Corresponding to: Kweon-Haeng Lee, Department of Pharmacology, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea. (Tel) 82-2-590-1203, (Fax) 82-2-3481-7602, (E-mail) Lkh@catholic.ac.kr

ABBREVIATIONS: NF2, Neurofibromatosis type 2; CRE, cyclic AMP-responsive element.

University, Korea). pCRE-Luc (4x) was purchased from Stratagene (La Jolla, CA, USA), and the pRL-TK-Luc and pCMV-Ras^{V12} were purchased from Promega (Madison, WI, USA) and BD Biosciences Clontech (Palo Alto, CA, USA), respectively.

Cell culture

The SK-N-BE(2)C human neuroblastoma cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (Gibco/BRL, Life Technologies Inc, CA, USA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C.

Transient transfection and transcription assay

The GenePorter2 transfection reagent (Gene Therapy Systems Inc, San Diego, CA, USA) was used for transfection according to the manufacturer's recommendation. For the transcriptional activation assay, the SK-N-BE(2)C cells (2.5×10^5) were plated on a 6-well plate (Corning Coaster, CA, USA). Sixteen hours later, the cells were transfected with 0.5 μg of the reporter plasmids along with the appropriate combination of the expression plasmids. The total amount of the transfected DNA was normalized, using the pcDNA3.1 plasmid as a carrier. Twenty-four hours later, transfected cells were harvested and the luciferase activity was analyzed using the Dual-luciferase assay system (Promega, Madison, WI, USA). For the forskolin treatment, the transfected cells were starved in a 0.1% FBS DMEM for another 24 hours, and the cells were then treated for 8 hours with 10 μM forskolin (Sigma) and the luciferase activity was measured. The results are expressed as the relative luciferase activities. The pRL-TK-Luc plasmid was co-transfected in order to normalize the variation in the transfection efficiency. The experiments were performed three times in duplicate.

Western blot analysis

The antibodies used were as follows: Anti-cyclin D1 (Neomarkers, Fremont, CA, USA), Anti-NF2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Anti-Ras (Upstate biotech, MA, USA) and Anti-β-actin (Sigma). For Western blot analysis, cells were rinsed with a phosphate-buffered saline (PBS) and lysed for 30 min on ice in a RIPA-B buffer (0.5% Nonidet P40, 20 mM Tris, pH 8.0, 50 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM dithiothreitol, 50 μg/ml phenylmethylsulfonyl fluoride). The insoluble material was removed by centrifugation at 12,000 rpm for 20 min at 4°C. The supernatant was then subjected to SDS-PAGE, which was followed by Western blot analysis. The blots were blocked in PBS with 5% skim milk and 0.05% Tween 20, followed by incubation with the appropriate primary antibodies, then with the secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology, CA, USA). The signals were visualized by the enhanced chemiluminescence system (Amersham phamacia biotech).

Reverse transcription-PCR analysis

The SK-N-BE(2)C cells were transfected with pcDNA-NF2 alone or together with pCMV-Ras^{V12}. The cells were harvested after 24 hours, and total RNA was isolated using a RNeasy kit (Qiagen Inc). One microgram of total RNA was subjected to reverse transcription-PCR analysis. First, Superscript II, RNase H reverse transcriptase was used to generate cDNAs using oligo (dT) 12~18 as primers according to the recommended conditions (Gibco/BRL, Life technologies Inc). One microliter of the cDNAs was mixed with 10 pmol of each primer specific to the cyclin D1 gene (5'-CTT CCT CTC CAA AAT GCC AG-3' and 5'-AGA GAT GGA AGG GGG AAA GA-3'). PCR amplification was performed for 25 cycles in a 20 μl-reaction mixture at 55°C as the annealing temperature. A control PCR using primers specific to the β-tubulin gene (5'-GTT GGT CTG GAA TTC TGT GAG-3'and 5'-AAG AAA TCC AAG CTG GAG TTC-3') was also carried out under the same conditions.

RESULTS

Merlin represses Ha-ras-mediated cyclin D1 expression

The well-known effects of Ras activation on the cell proliferation involve the cyclin D1 accumulation at the transcriptional level. And, it was reported that merlin inhibits the Ha-*ras*-induced anchorage-independent cell growth (Tikoo et al, 1994). Therefore, it was of interest to test whether merlin was involved in inhibiting the Ras-mediated cyclin D1 expression in SK-N-BE(2)C cells. Thus, the SK-N-BE(2)C cells were transfected with the Ha-*ras* expression plasmid with or without the merlin expression plasmid, and the cell lysates were subsequently subjected to either Western blot analysis with anti-cycin D1 antibody (Fig. 1A) or RT-PCR (Fig. 1B). As shown in Fig. 1A, cyclin D1 level was induced by the overexpression of Ha-*ras*, which was abolished by the co-expression of merlin. The same pattern of result was obtained in the same set of experiment for the cyclin D1 mRNA level (Fig.

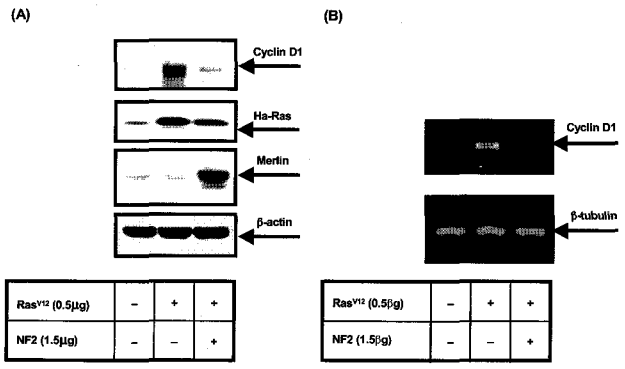

Fig. 1. Effect of merlin on the Ha-*ras*-mediated cyclin D1 expression. (A) The SK-N-BE(2)C cells were transiently transfected with an expression vector for active Ras alone or together with a vector encoding the full-length merlin. Twenty-four hours after transfection, the lysates of SK-N-BE(2)C cells were analyzed for cyclin D1, Ras and merlin proteins by Western blotting. The amount of β-actin was assayed as a loading control. (B) The diagram shows the reverse transcription-PCR (RT-PCR) results for cyclin D1 and β-tubulin mRNAs as an interal control. The total RNA isolated from SK-N-BE(2)C cells, transfected as descrided in Fig. 1A, was subjected to the RT-PCR.

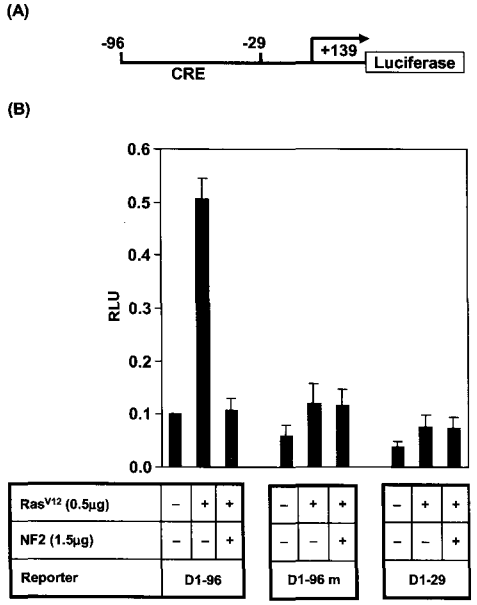

Fig. 2. Effect of merlin on the CRE-dependent transcription of the cyclin D1 promoter. (A) Schematic representation of the cyclin D1 promoter (D1-96, -29) is shown. Factor binding site for CREB is indicated. (B) Inhibition of the Ras-induced cyclin D1 promoter activation by merlin. The D1-96m contains three-point mutation (TAACGTCA to TAAgaTCt) in the CRE site. The D1-29 does not contain CRE site. The 0.5 μg of each cyclin D1 promoter plasmid (D1-96, D1-96m and D1-29) was transfected into the SK-N-BE(2)C cells, along with 0.5 μg of the expression plasmid for Ras^{V12} (pCMV-Ras^{V12}) and 1.5 μg of the merlin expression plasmid (pcDNA-NF2) as indicated. After 24 hours, the cell lysates were prepared and the luciferase activity was determined. The experiments were performed three times in duplicate. The standard errors are shown on the top of each bar. The numbers in the left column represent the relative luciferase activity.

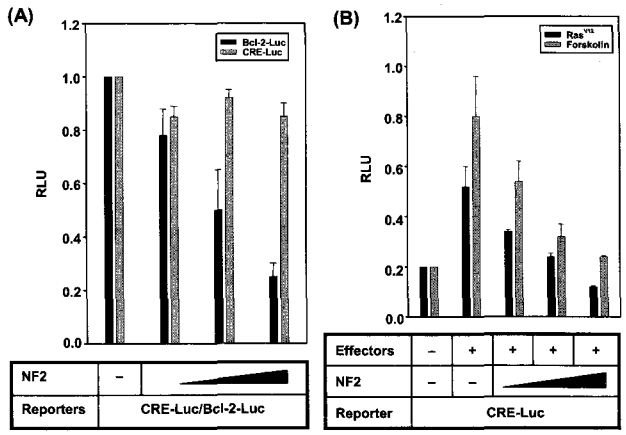

Fig. 3. Effect of merlin on the consensus CRE-dependent transcription. (A) The 0.5 μg of CRE-Luc or Bcl-2-Luc reporter plasmid was transfected into the SK-N-BE(2) cells, with 0, 0.1, 0.5, or 1.0 μg of the merlin expression plasmid, pcDNA-NF2. The Bcl-2 promoter activity was examined as a negative control. After 24 hours, the cell lysates were prepared and the luciferase activity was determined. (B) The 0.5 μg of CRE-Luc reporter plasmid was transfected into the SK-N-BE(2)C cells, along with 0, 0.1, 0.5, or 1.0 μg of the merlin expression plasmid, pcDNA-NF2. As effectors for the CRE-Luc, the reporter was either co-transfected with the 0.5 μg of the Ras^{V12} expression plasmid or treated with 10 μM forskolin for 8 hours after transfection. The experiments were performed three times in duplicate, and the results were averaged. The standard errors are shown on the top of each bar. The numbers in the left column represent the relative luciferase activity.

1B). These results suggest that merlin down-regulates cyclin D1 expression at the transcriptional level by inhibiting the Ras activity.

Merlin represses the cyclin D1 promoter activity induced through the cyclic AMP-responsive element

The cyclic AMP-responsive element (CRE), located upstream of the mRNA start site, plays a key role in both the basal and induced cyclin D1 expression (Beier et al, 1999; Lee et al, 1999; D'Amico et al, 2000; Nagata et al, 2001). A previous report showed that the active Ras induced the cyclin D1 promoter activity through the CRE (Tetsu & McCormick, 1999). Therefore, the merlin effect was tested on the CRE-mediated cyclin D1 promoter activity in SK-N-BE(2)C cells. The cyclin D1 promoter construct encompassing the -96 region from the transcription start site (D1-96) was used (Fig. 2A). This region contains the known CRE. The D1-96 m construct with the mutant CRE (TAACGTCA → TAAgaTCt) and the D1-29 construct encompassing the -29 region were used as controls. Fig. 2B shows that the active Ras induced the D1-96 about 5 folds and the co-expression of merlin repressed it to the basal level. The active Ras induced D1-96m or D1-29 just about two folds each, and it was not affected by the co-expression of merlin. The similar pattern of results to D1-96 were obtained with the full length cyclin D1 promoter (D1-973) (data not shown). Overall, these results show that merlin inhibits the function of Ras on the CRE-mediated cyclin D1 promoter activation.

Merlin inhibits consensus CRE-dependent promoter activity

The effect of merlin was examined directly on the consensus CRE-dependent promoter activity (CRE-Luc). As shown in Fig. 3A, the basal CRE activity was reduced by merlin in a dose-dependent manner in SK-N-BE(2)C cells. In contrast, no dose dependency of merlin was observed with the Bcl-2 promoter reporter, suggesting that merlin specifically inhibits the CRE-regulated promoter activity in unstimulated cells.

Then, we investigated the merlin activity on the CRE-dependent promoter activity. As shown in Fig. 3B, the CRE-Luc activity was stimulated about 2.5 fold by the active Ras and 4 folds by forskolin (10 μM), an activator of CREB (Muller et al, 2001). The co-expression of merlin repressed these increased CRE-Luc activity in a dose-dependent manner. These results suggest that merlin can inhibit both the endogenous and the CRE-dependent transcription increased by exogenous stimuli.

DISCUSSION

Little is known about the molecular mechanisms underlying the function of NF2 as a tumor. Recent studies showed that the Rac1/JNK and ERK 1/2 pathways are up-regulated

in primary human schwannoma and MEF cells lacking merlin protein (Kaempchen et al, 2003; Lallemand et al, 2003). They also showed that merlin is important in reducing the cyclin D1 expression level. However, the mechanism of the decrease in the cyclin D1 level was not elucidated. This report for the first time suggested a molecular mechanism of merlin mediated repression of cyclin D1 expression in association with the Ras function: Merlin repressed the cyclin D1 promoter activity induced by Ras, and interestingly, this appeared to be mediated through the inhibition of the CRE-dependent cyclin D1 promoter activity. In addition, we showed that merlin could repress forskolin-induced CRE activity, suggesting that merlin has negative effects on the PKA-dependent signaling pathway. It coud be possible that the CREB/ATF-2 phosphorylation or CRE occupancy might be down-regulated by merlin.

The putative CRE site is a major mediator of the induction of the cyclin D1 promoter by the binding of CREB/ATF-2 and c-Jun heterodimer complex (Herber et al, 1994; Sabbah et al, 1999). However, due to the complexity in the regulation of the cyclin D1 promoter, it is possible that multiple factors bind to the CRE/ATF-2 site simultaneously by the Ras signaling (Watanabe et al, 1996; Brown et al, 1998). Therefore, the cross-talk between the Ras signaling and cyclin D1 may be dependent on the cell context such as the abundance and availability of the preferential trans-acting factors. On the other hand, recent studies have shown that Ras is able to induce the cyclin D1 expression during the G2 phase by altering the translational efficiency of preformed cyclin D1 mRNA (Guo et al, 2002; Sa et al, 2002). Therefore, we could not completely exclude the possibility that merlin is involved in the post-transcriptional regulation of cyclin D1 expression by inhibiting the Ras signaling pathway.

Previously, we have shown that merlin inhibits the Ras-mediated AP-1 activity and SRE-dependent transcription as well as the TNF-α-mediated NF-kappa B activation (Kim et al, 2002a; Kim et al, 2002b; Lim et al, 2003). Together with these earlier studies, the present study suggests that merlin interferes with the Ras signaling pathway, thereby down-regulating its mitogenic activities. The inhibition of Ras activity by merlin could be one of the mechanisms for the merlin-mediated cell growth inhibition. Further experiments are needed to determine if merlin plays a role in the regulation of the cyclin D1 gene by inhibiting other oncogenic signals.

ACKNOWLEDEMENT

The authors wish to thank the other members of this laboratory for their much needed assistance. And we thank Dr. Gerard Redeuilh and Dr. David Gutmann for cyclin D1 reporter plasmids and pcDNA-NF2 expression vector, respectively. This work was supported by grants from The Korean Ministry of Health and Welfare (HMP-00-GN-01-0002).

REFERENCES

Beier F, Lee RJ, Taylor AC, Pestell RG, LuValle P. Identification of the cyclin D1 gene as a target of activating transcription factor 2 in chondrocytes. *Proc Natl Acad Sci USA* 96: 1433–1438, 1999

Boguski MS, McCormick F. Proteins regulating Ras and its relatives. *Nature* 366: 643–654, 1993

Bretscher A, Chambers D, Nguyen R, Reczek D. ERM-Merlin and EBP50 protein families in plasma membrane organization and function. *Annu Rev Cell Dev Biol* 16: 113–143, 2000

Brown JR, Nigh E, Lee RJ, Ye H, Thompson MA, Saudou F, Pestell RG, Greenberg ME. Fos family members induce cell cycle entry by activating cyclin D1. *Mol Cell Biol* 18: 5609–5619, 1998

D'Amico M, Hulit J, Amanatullah DF, Zafonte BT, Albanese C, Bouzahzah B, Fu M, Augenlicht LH, Donehower LA, Takemaru K, Moon RT, Davis R, Lisanti MP, Shtutman M, Zhurinsky J, Ben-Ze'ev A, Troussard AA, Dedhar S, Pestell RG. The integrin-linked kinase regulates the cyclin D1 gene through glycogen synthase kinase 3beta and cAMP-responsive element-binding protein-dependent pathways. *J Biol Chem* 275: 32649–32657, 2000

Feig LA and Cooper GM. Inhibition of NIH 3T3 cell proliferation by a mutant ras protein with preferential affinity for GDP. *Mol Cell Biol* 8: 3235–3243, 1988

Filmus J, Robles AI, Shi W, Wong MJ, Colombo LL, Conti CJ. Induction of cyclin D1 overexpression by activated ras. *Oncogene* 9: 3627–3633, 1994

Gronholm M, Sainio M, Zhao F, Heiska L, Vaheri A, Carpen O. Homotypic and heterotypic interaction of the neurofibromatosis 2 tumor suppressor protein merlin and the ERM protein ezrin. *J Cell Sci* 112: 895–904, 1999

Guo Y, Stacey DW, Hitomi M. Post-transcriptional regulation of cyclin D1 expression during G2 phase. *Oncogene* 21: 7545–7556, 2002

Gutmann DH, Sherman L, Seftor L, Haipek C, Hoang Lu K, Hendrix M. Increased expression of the NF2 tumor suppressor gene product, merlin, impairs cell motility, adhesion and spreading. *Hum Mol Genet* 8: 267–275, 1999

Herber B, Truss M, Beato M, Muller R. Inducible regulatory elements in the human cyclin D1 promoter. *Oncogene* 9: 1295–1304, 1994

Ikeda K, Saeki Y, Gonzalez-Agosti C, Ramesh V, Chiocca EA. Inhibition of NF2-negative and NF2-positive primary human meningioma cell proliferation by overexpression of merlin due to vector-mediated gene transfer. *J Neurosurg* 91: 85–92, 1999

Kaempchen K, Mielke K, Utermark T, Langmesser S, Hanemann CO. Upregulation of the Rac1/JNK signaling pathway in primary human schwannoma cells. *Hum Mol Genet* 12: 1211–1221, 2003

Kim H, Lim JY, Kim YH, Kim H, Park SH, Lee KH, Han H, Jeun SS, Lee JH, Rha HK. Inhibition of ras-mediated activator protein 1 activity and cell growth by merlin. *Mol Cells* 14: 108–114, 2002a

Kim JY, Kim H, Jeun SS, Rha SJ, Kim YH, Ko YJ, Won J, Lee KH, Rha HK, Wang YP. Inhibition of NF-kappaB activation by merlin. *Biochem Biophys Res Commun* 296: 1295–1302, 2002b

Lallemand D, Curto M, Saotome I, Giovannini M, McClatchey AI. NF2 deficiency promotes tumorigenesis and metastasis by destabilizing adherens junctions. *Genes Dev* 17: 1090–1100, 2003

Lee RJ, Albanese C, Stenger RJ, Watanabe G, Inghirami G, Haines GK 3rd, Webster M, Muller WJ, Brugge JS, Davis RJ, Pestell RG. pp60 (v-src) induction of cyclin D1 requires collaborative interactions between the extracellular signal-regulated kinase, p38, and Jun kinase pathways. A role for cAMP response element-binding protein and activating transcription factor-2 in pp60 (v-src) signaling in breast cancer cells. *J Biol Chem* 274: 7341–7350, 1999

Lim JY, Kim H, Kim YH, Kim SW, Huh PW, Lee KH, Jeun SS, Rha HK, Kang JK. Merlin suppresses the SRE-dependent transcription by inhibiting the activation of Ras-ERK pathway. *Biochem Biophys Res Commun* 302: 238–245, 2003

Liu JJ, Chao JR, Jiang MC, Ng SY, Yen JJ, Yang-Yen HF. Ras transformation results in an elevated level of cyclin D1 and acceleration of G1 progression in NIH 3T3 cells. *Mol Cell Biol* 15: 3654–3663, 1995

Mechta F, Lallemand D, Pfarr CM, Yaniv M. Transformation by ras modifies AP1 composition and activity. *Oncogene* 14: 837–47, 1997

Morrison H, Sherman LS, Legg J, Banine F, Isacke C, Haipek CA, Gutmann DH, Ponta H, Herrlich P. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. *Genes Dev* 15: 968–980, 2001

Mulcahy LS, Smith MR, Stacey DW. Requirement for ras proto-oncogene function during serum-stimulated growth of NIH 3T3 cells. *Nature* 241: 241–243, 1985

Muller FU, Boknik P, Knapp J, Linck B, Luss H, Neumann J, Schmitz W. R Activation and inactivation of cAMP-response element-mediated gene transcription in cardiac myocytes. *Cardiovasc Res* 52: 95–102, 2001

Nagata D, Suzuki E, Nishimatsu H, Satonaka H, Goto A, Omata M, Hirata Y. Transcriptional activation of the cyclin D1 gene is mediated by multiple cis-elements, including SP1 sites and a cAMP-responsive element in vascular endothelial cells. *J Biol Chem* 276: 662–669, 2001

Robles AI, Rodriguez-Puebla ML, Glick AB, Trempus C, Hansen L, Sicinski P, Tennant RW, Weinberg RA, Yuspa SH, Conti CJ. Reduced skin tumor development in cyclin D1-deficient mice highlights the oncogenic ras pathway in vivo. *Genes Dev* 12: 2469–2474, 1998

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B, et al. Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature* 363: 515–521, 1993

Sa G, Hitomi M, Harwalkar J, Stacey AW, GC GC, Stacey DW. Ras is active throughout the cell cycle, but is able to induce cyclin D1 only during G2 phase. *Cell Cycle* 1: 50–58, 2002

Sabbah M, Courilleau D, Mester J, Redeuilh G. Estrogen induction of the cyclin D1 promoter: involvement of a cAMP response-like element. *Proc Natl Acad Sci USA* 96: 11217–11222, 1999

Shaw RJ, Paez JG, Curto M, Yaktine A, Pruitt WM, Saotome I, O'Bryan JP, Gupta V, Ratner N, Der CJ, Jacks T, McClatchey AI. The NF2 tumor suppressor, merlin, functions in Rac-dependent signaling. *Dev Cell* 1: 63–72, 2001

Sherman L, Xu HM, Geist RT, Saporito-Irwin S, Howells N, Ponta H, Herrlich P, Gutmann DH. Interdomain binding mediates tumor growth suppression by the NF2 gene product. *Oncogene* 15: 2505–2509, 1997

Smith MR, De Gudicibus SJ, Stacey DW. Requirement for c-ras proteins during viral oncogene transformation. *Nature* 320: 540–543, 1986

Tetsu O and McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398: 422–426, 1999

Thomas G, Merel P, Sanson M, Hoang-Xuan K, Zucman J, Desmaze C, Melot T, Aurias A, Delattre O. Neurofibromatosis type 2. *Eur J Cancer* 30A: 1981–1987, 1994

Tikoo A, Varga M, Ramesh V, Gusella J, Maruta H. An anti-Ras function of neurofibromatosis type 2 gene product (NF2/ Merlin). *J Biol Chem* 269: 23387–23390, 1994

Tsukita S, Oishi K, Sato N, Sagara J, Kawai A, Tsukita S. ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J Cell Biol* 126: 391–401, 1994

Watanabe G, Howe A, Lee RJ, Albanese C, Shu IW, Karnezis AN, Zon L, Kyriakis J, Rundell K, Pestell RG. Induction of cyclin D1 by simian virus 40 small tumor antigen. *Proc Natl Acad Sci USA* 93: 12861–12866, 1996