Mechanism of E1A-Induced Transforming Growth Factor-β (TGF-β) Resistance in Mouse Keratinocytes Involves Repression of TGF-β Type II Receptor Transcription*

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Cellular transformation driven by the E1A oncogene is associated with the development of cellular resistance to the growth inhibitory effects of transforming growth factor-β (TGF-β). We demonstrate that development of resistance occurs simultaneously with decreased expression of TGF-β type II receptor (TGF-β RII) mRNA and protein. To determine whether changes in transcriptional regulation are responsible for the decreased receptor expression in E1A-transformed cells, a series of mobility shift assays was performed utilizing nuclear extracts from E1A-transformed and untransformed murine keratinocytes using radiolabeled positive regulatory elements (PRE1 and PRE2) of the TGF-β RII promoter. The results from these assays suggest that E1A-transformed cells express markedly lower levels of nuclear proteins that bind specifically to PRE1 and PRE2. Transfection of both E1A-transformed and untransformed cell lines with a series of mutant promoter constructs confirmed that both PREs contribute significantly to basal expression of TGF-β RII and that inactivation of either element leads to markedly reduced promoter activity. We conclude that development of TGF-β resistance in E1A-transformed cells is achieved in part through transcriptional down-regulation of the TGF-β RII gene and that this down-regulation is the result of decreased expression of unidentified transcription factor complexes that interact with PRE1 and PRE2.

Transforming growth factor-β (TGF-β) is considered the prototypical multifunctional cytokine, playing a central role in various vital cellular processes such as growth, differentiation, synthesis of matrix components, and apoptosis (1–4). Appropriate cellular responses to extracellular TGF-β depend on a system of multiple TGF-β-specific cell surface receptor proteins and are initiated with binding of TGF-β ligand directly to the TGF-β type II receptor, which is a constitutively active serine-threonine kinase (5–8). When bound to ligand, the type II receptor forms a heteromeric complex with the TGF-β type I receptor, which then leads to phosphorylation and activation of the type I receptor serine-threonine kinase and allows intracellular signaling to proceed.

One of the most prominent effects of TGF-β in vitro is pronounced inhibition of growth of epithelial cells (9). However, it is well known that epithelial-derived cancers typically demonstrate resistance to the growth inhibitory effect of TGF-β (10). Acquisition of such TGF-β resistance by clonal populations of tumor cells may represent an essential step in the process of carcinogenesis. Preliminary evidence indicates that TGF-β resistance develops relatively late in the evolution of cancer, coinciding with a period when tumor phenotype first becomes recognizably malignant (11).

Sporn and Roberts (12) first predicted that receptor defects would be identified as the basis of TGF-β resistance, a prediction soon confirmed by the finding of a strong correlation between structural abnormalities of the TGF-β RII gene and development of TGF-β resistance in human gastric cancer cell lines (13). Subsequently, a specific TGF-β RII mutation has been identified and associated with defective DNA mismatch repair in colon cancer cells from patients with the HNPCC (hereditary nonpolyposis colon cancer) syndrome (14). A similar association has been found in gastric cancer as well (15).

It is becoming clear that the TGF-β signaling pathway sub-serves a vital tumor suppressor function in various cell lines (16, 17). However, we have identified several TGF-β-resistant cancer cell lines, which express decreased levels of cell surface receptors and yet contain no recognizable mutations in the TGF-β receptor genes. This observation has suggested to us the possibility that transcriptional regulation or posttranslational mechanisms may lead to development of TGF-β resistance in some cases.

A high level of structural complexity for the promoter region of TGF-β RII enhances the potential for significant transcriptional regulation of this gene. Preliminary characterization of the promoter region of TGF-β RII has revealed the presence of two positive regulatory elements (PRE1 and PRE2) and at least one negative regulatory element (NRE) in addition to the core promoter element (18). Discrete target sequences have been identified within each of these transcriptional regulatory elements, and each appears to interact with multiple nuclear proteins, including six putative novel transcription factor complexes.

Cellular transformation driven by the E1A oncogene is a popular system for modeling the genetic events of carcinogenesis. This model was first introduced in 1962 when it was discovered that adenovirus infection leads to sarcoma formation in newborn hamsters (19). Since then, considerable effort has been directed toward dissecting the genetic events associated with the development of malignancy in this system (20–25). The adenovirus genome has been completely sequenced,

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* The abbreviations used are: TGF-β, transforming growth factor-β; RII, type II receptor; PRE, positive regulatory element; NRE, negative regulatory element; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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and numerous separable genetic functions have been identified and categorized as either 'early' or 'late' according to their time of expression relative to the onset of DNA replication (26). Expression of one of the adenovirus early region genes known as E1A is required for cellular transformation in vitro and has also been associated with several other cellular effects including induction of resistance to growth inhibition by TGF-β.

To investigate the mechanism underlying development of E1A-mediated TGF-β resistance, a series of experiments was performed utilizing a dl799N mouse keratinocyte cell line stably transfected with an E1A expression vector (27). A second mouse keratinocyte cell line transfected with an enhancerless SV2 promoter was used as a control. Northern blot analysis demonstrated a marked decrease in TGF-β RII mRNA expression from E1A-transfected dl799N cells compared with control. Receptor cross-linking assay further revealed that decreased mRNA levels were associated with diminished expression of TGF-β RII protein. Chloramphenicol acetyltransferase transfection assays demonstrated that the regulatory activity of PRE1, PRE2, and NRE was intact in dl799N cells although levels of transcription were consistently lower than in SV2neo cells transfected with the same constructs. Electrophoretic mobility shift assays revealed a significant reduction in DNA binding by the transcription factor complexes interacting with both PRE1 and PRE2. This study suggests that TGF-β resistance in E1A-transformed mouse keratinocytes is mediated in part by transcriptional down-regulation of the TGF-β RII gene and that this negative regulation occurs at both the PRE1 and PRE2 loci.

MATERIALS AND METHODS

Cell Culture—Pam212(SV2neo) and Pam212(dl799N) mouse keratinocyte cell lines were obtained from Paolo Dott (27). Pam212(SV2neo) cells have been stably transfected with an SV2 promoter and neomycin resistance gene construct. Pam212(dl799N) cells have been stably transfected with an E1A expression vector. Both cell lines were maintained in Dulbecco’s modified Eagle’s medium containing low glucose, 10 mM Hepes, pH 7.4, and 1 mg/ml bovine serum albumin fraction V. Binding was carried out with 100 µl 32P-labeled TGF-β in the presence and absence of 100-fold molar excess of unlabeled TGF-β, and cells were incubated on a rotating platform at 4°C for 2.5 h. Cells were washed twice with cold wash buffer containing 1 x minimal essential medium, 25 mM Hepes, pH 7.4, and 1 mg/ml bovine serum albumin fraction V. Binding was carried out with 100 µl 32P-labeled TGF-β in the presence and absence of 100-fold molar excess of unlabeled TGF-β, and cells were incubated on a rotating platform at 4°C for 2.5 h. Cells were washed twice with cold wash buffer containing 1 x minimal essential medium, 25 mM Hepes, pH 7.4, and 1 mg/ml bovine serum albumin fraction V. Binding was carried out with 100 µl 32P-labeled TGF-β in the presence and absence of 100-fold molar excess of unlabeled TGF-β, and cells were incubated on a rotating platform at 4°C for 2.5 h.

Results—Northern blot analysis was utilized to analyze mRNA expression levels for the TGF-β RII and TGF-β1 genes. Fig. 1 shows that expression of TGF-β RII mRNA is significantly lower in E1A-transformed dl799N cells compared with the control SV2neo cells. In contrast, expression of TGF-β1 mRNA is equivalent between the two cell lines. GAPDH mRNA was used to control for sample loading and indicates that the sample quantity was slightly greater for dl799N indicating that the reduction in TGF-β RII mRNA expression was even greater than that visualized.

RNA was isolated from cell samples treated with 1 nM retinoic acid, 1 nM 1,25-(OH)2D3, or a combination of both retinoic acid and 1,25-(OH)2D3. A previous study involving human myeloid leukemia cells reported an increase in expression of the TGF-β type II receptor following treatment with retinoic acid (30). Retinoic acid and/or 1,25-(OH)2D3, treatment of the murine keratinocyte cell lines used in our study, however, had no apparent effect on the level of TGF-β1 or TGF-β RII mRNA expression.
TGF-β was performed to determine whether decreased mRNA levels for TGF-β RII translated into decreased protein expression. Receptor cross-linking was performed three times, and Fig. 2 shows representative results. Levels of TGF-β type III and type I receptor cross-linked protein were minimally decreased in the E1A-transformed dl799N cells compared with control. The level of TGF-β type II receptor protein, however, was markedly decreased.

Transcriptional Activity of the TGF-β RII Promoter Is Reduced in dl799N Cells—In order to determine the functional activity of the two reported positive regulatory elements in the TGF-β RII promoter region, progressively shorter fragments of the 5′-flanking region fused with the coding region of the bacterial CAT gene in the plasmid pGEM-SV0CAT were transfected into both SV2neo and dl799N cells. As seen in Fig. 3, transcriptional activity was less in dl799N cells than in SV2neo cells for each construct tested. In both cell lines, deletion of the sequence between −1000 and −274 led to a marked increase in transcriptional activity, suggesting the presence of an as yet uncharacterized negative regulatory element upstream of PRE1. Deletion of the sequence between −274 and −137 removed PRE1 and led to a predictable decrease in CAT activity. Deletion of nucleotides −137 to −47 eliminated the sequence for a putative NRE (18) and led to a marked increase in transcription levels. Deletion of the core promoter element between −47 and +2, as expected, significantly decreased transcriptional activity. A relatively high level of CAT activity remained with transfection of the smallest construct, +2 to +50, indicating that the second positive regulatory element, PRE2, was active in both cell lines. Transcriptional activity was consistently higher in SV2neo cells compared with the E1A-transformed dl799N cells, varying from three to more than six times greater. Regulatory activity of PRE1, PRE2, and NRE appears to be intact in both E1A-transformed and control cell lines.

Identification of a Single DNA Binding Protein from SV2neo Cells That Interacts with PRE1 Target Sequence—Previous characterization of the TGF-β RII promoter region identified two discrete DNA binding protein complexes, an AP1/CREB-like transcription factor complex and a putative novel transcription factor complex. To determine whether these two protein complexes were similarly present in mouse keratinocyte cells, we synthesized a series of mutant oligonucleotides derived from PRE1 (Fig. 4A). Each mutant oligonucleotide contained a 4-base pair substitution mutation. EMSA was then performed using a radiolabeled PRE1 probe incubated with SV2neo nuclear extract in competition with the series of mutant oligonucleotides. As shown in Fig. 4B, specific binding was observed with a single protein complex that migrated at the same position as complex a, the previously identified AP1/CREB-like transcription factor complex. Competitive binding was abolished by mutation of the complex a target sequence.
confirm the identity of the single protein complex, another EMSA was performed with radiolabeled PRE1 oligonucleotide probe incubated with SV2neo nuclear extract in competition with double-stranded oligonucleotides representing the consensus sequences for several known transcription factors. Only the unlabeled AP1 and CRE sequences demonstrated competition with the labeled PRE1 probe for binding to the protein complex, indicating that it represents the AP1/CRE-like transcription factor (data not shown). Complex b (18) interactions were extremely low or undetectable.

Expression of E1A Is Associated with Decreased Interaction of Complex “a” with PRE1—To determine whether E1A expression affects interaction of transcription factor complex a with PRE1, another EMSA was performed utilizing a labeled PRE1 probe and nuclear extract from both dl799N and SV2neo cells. As shown in Fig. 5, interaction of complex a with PRE1 is markedly reduced in the E1A-expressing dl799N cell line compared with control. Lanes 1 and 3 represent reaction mixtures containing 5’ random-labeled oligonucleotide representing PRE1 (~219 to ~172) incubated with 10 μg of purified SV2neo or dl799N nuclear extract, respectively. Lanes 2 and 4 demonstrate specificity of binding and represent identical reaction mixtures in competition with unlabeled PRE1 oligonucleotide. The level of activity represented by the minor bands is equivalent in both cell lines, demonstrating equal loading of protein. Semiquantitative analysis of the absolute levels of activity represented by the individual bands in this assay using a scintillation counter suggests a relative decrease of approximately 5-fold, which correlates well with the observed decrease in TGF-β RII transcriptional activity in E1A-expressing cells. However, direct quantitative comparisons of DNA-protein complex binding and transcriptional activity should be cautiously interpreted given the likelihood of additional factors influencing overall levels of transcription.

Identification of Three Nuclear Protein Complexes from SV2neo Cells Interacting with PRE2—We have previously described three discrete nuclear protein complexes that interact specifically with target sequences within PRE2. To demonstrate that the mouse keratinocyte cell line SV2neo expressed the same complement of proteins, we synthesized another series of mutant oligonucleotides based on the PRE2 DNA sequence (Fig. 6A). Each mutant oligonucleotide contained a 5-base pair substitution mutation. EMSA was performed using a 32P-labeled PRE2 double-stranded oligonucleotide probe incubated with SV2neo nuclear extract in competition with each mutant oligonucleotide. As shown in Fig. 6B, lane 1, three major protein complexes were observed in the absence of competing oligonucleotides. Mutation of nucleotides +16 to +20 (AAGTG, M4) led to decreased competition for binding to complex c, while competition for binding to complexes d and e was diminished by mutations through a longer sequence from +11 to +29 (AGTTTCTGTGTTCG, M3-M6). These target sequences are equivalent to those previously identified (Figs. 6B and 7) in other cell lines.

Expression of E1A Is Associated with Decreased Interaction of Complexes “c,” “d,” and “e” with PRE2—Another EMSA was performed using a 32P-labeled PRE2 oligonucleotide probe and nuclear extract from both dl799N and SV2neo cells. Fig. 7 shows that binding of all three complexes, a, b, and c, to PRE2 is significantly reduced in E1A-expressing dl799N cells compared with control. Lanes 1 and 3 contain reaction mixtures consisting of 5’ random-labeled oligonucleotide representing PRE2 (+1 to +50) incubated with 10 μg of purified SV2neo or dl799N nuclear extract, respectively. Lanes 2 and 4 contained identical reaction mixtures in competition with unlabeled PRE2 oligonucleotide to demonstrate specificity of binding.
Press all three complexes as seen by the binding patterns of the three distinct transcription factor complexes. Mouse keratinocytes express PRE2 showing location of previously reported target sequences for neo oligonucleotide incubated with SV2 transformed cells, we created a series of CAT constructs containing intact PRE1 and PRE2 oligonucleotide incubated with SV2neo cell nuclear extract in competition with mutant oligonucleotides from A, C, wild-type sequence of PRE2 showing location of previously reported target sequences for three distinct transcription factor complexes. Mouse keratinocytes express all three complexes as seen by the binding patterns of the three bands demonstrated in B.

Activity of the rapidly migrating minor band in lanes 1 and 3 is equivalent, indicating equivalent protein loading.

**PRE1 and PRE2 Transcriptional Regulatory Activity Is Down-regulated in Cells Expressing E1A**—To compare the activity of PRE1 and PRE2 in both E1A-transformed and untransformed cells, we created a series of CAT constructs containing various combinations of mutations and deletions in the X and Y target sequences of PRE1 as well as the Z target sequence of PRE2. Fig. 8 presents a schematic representation of the constructs used. In each case, presence of a bar indicates that the wild-type sequence is intact while absence signifies that the sequence has been mutated. These constructs were transfected into both SV2neo and dl799N cells, and CAT activity was assayed as a measure of transcriptional activity. Fig. 8 shows that basal transcriptional activity of the TGF-β RII promoter in both cell lines is principally dependent on the Y target sequence on PRE1 and the Z target sequence on PRE2. A comparison of the activity of constructs −219/+35 and −219M7/+35 reveals that mutation of the Y sequence markedly reduces measured CAT activity following transfection into both the SV2neo control keratinocyte cell line as well as the dl799N E1A-transformed cell line. Similarly, comparing −219/ +35 to −219/+35M3 demonstrates that mutation of the Z sequence leads to an equally sharp decrease in basal transcription rates in both cell lines. In contrast, mutation of the X target sequence, which interacts with a binding protein that appears not to be expressed in mouse keratinocytes, failed to inhibit transcriptional activity to any significant degree (compare activity of construct −219/+35 with construct −219M5/+35).

The highest level of transcriptional activity was obtained following transfection of constructs containing intact Y and Z target sequences (constructs −219/+35 and −219M5/+35), and conversely, the lowest level of activity was observed with the use of constructs containing mutations or deletions of the Y and Z target sequences (constructs −219M7/+35M3 and −219M7/ +2). Overall transcriptional activity was markedly reduced in E1A-transformed cells (range from 0.6 to 5.2% acetylation) compared with control (range from 1.3 to 23.5% acetylation). However, the pattern of CAT activity was identical for the two different cell lines, indicating that both PRE1 and PRE2 regulatory elements are functional in dl799N cells despite their lower activity levels.

**DISCUSSION**

A growing body of experimental evidence supports the concept that TGF-β RII operates as a tumor suppressor gene (16, 17). Expression of TGF-β RII is required for the growth inhibitory effects of TGF-β ligands on proliferating epithelial cells. Mutation of the TGF-β RII gene has been observed in a number of different human malignancies, including colon, gastric, and endometrial cancers, and is highly correlated with the development of TGF-β resistance (15). Transfecting human breast cancer and colon cancer as well as hepatoma cells lacking type II receptor with wild type TGF-β type II receptor restores sensitivity to TGF-β and decreases tumorigenicity in transplanted breast and colon cancer cells (31, 32). Moreover, transfection of antisense TGF-β1 into a TGF-β-sensitive FET colon cancer cell line enhances its tumorigenicity (33).

Similarly, numerous studies indicate that transcriptional regulation makes an important contribution to determining the level of expression of TGF-β RII. Transfection of TGF-β-sensitive murine myeloid cells with the src oncogene results in development of TGF-β resistance, which correlates with de-
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Increased expression of TGF-β RII protein and mRNA (34). Conversely, transfection of TGF-β-resistant esophageal epithelial cells with cyclin D1 leads to enhanced expression of TGF-β RII mRNA and protein along with increased TGF-β sensitivity (35). Our previous study of the TGF-β RII promoter reported the presence of two separate positive regulatory elements in addition to the core promoter element (18), and we have since confirmed the presence of at least two additional negative regulatory elements (35). Each regulatory element interacts with multiple nuclear DNA binding proteins, including several putative novel transcription factors, in a sequence-specific manner. Such a complex array of interdependent regulatory elements and binding proteins easily allows for the possibility of multiple parallel transcriptional regulatory pathways.

Our findings, as presented in the current study, demonstrate that transformation of keratinocytes with the E1A oncoprotein is associated with decreased expression of TGF-β RII protein and an absolute reduction in steady-state levels of TGF-β mRNA resulting from down-regulation of TGF-β RII promoter activity. Specific binding of nuclear protein complexes to the first and second positive regulatory elements of TGF-β RII is markedly decreased in E1A-transformed cells compared with control, suggesting that transcription of TGF-β RII is being repressed at the level of transcription factor expression or DNA interaction. Quantitatively, the decrease in TGF-β RII transcript level demonstrated in Fig. 1 and the lower cell-surface expression of TGF-β RII shown in Fig. 2 may not appear to correlate with the 3- to 6-fold decrease in transcriptional activity from the reporter construct revealed in Fig. 3. The influence of additional uncharacterized enhancer- and/or repressor-like elements on the specific cellular expression levels of TGF-β RII cannot be ruled out. Moreover, absolute levels of transcript and protein are likely influenced by additional variably active cellular processes that make a direct quantitative analysis impossible.

Similar transfection experiments have been performed utilizing SNU gastric cancer cells resistant to growth inhibition by TGF-β. Preliminary data suggest that PRE2 may have a more significant role in determining TGF-β RII transcription levels in SNU cells (34). In this study, it also appears as if the interaction of complexes c, d, and e with PRE2 demonstrate a greater overall decrease compared with the interaction of complex a and PRE1. Moreover, Fig. 8 reveals that the greatest difference in transcriptional activity occurs when PRE2 alone is altered. These data suggest that the nuclear factors interacting with PRE2 may constitute the more important targets in transcriptional regulation of TGF-β RII expression.

A previous study by Missero et al. (27) also examined the phenomenon of TGF-β resistance resulting from transformation of keratinocytes with the E1A oncoprotein. Their data revealed that development of resistance to TGF-β growth inhibition most closely correlated with binding of E1A proteins to the retinoblastoma gene product, pRb, and three other unidentified cellular proteins, p60, p107, and p300. These results led them to conclude that TGF-β resistance in cells transformed with E1A developed via a postreceptor mechanism involving negative interactions with downstream elements of the TGF-β signaling pathway.

Although it is conceivable that a postreceptor mechanism also contributes to the observed decrease in TGF-β sensitivity that occurs in E1A-transformed cells, our data strongly support the existence of an additional mechanism that results in direct transcriptional down-regulation of TGF-β type II receptor expression as well. Moreover, our finding that E1A expression results in decreased binding of specific nuclear proteins to the TGF-β RII promoter suggests an alternative interpretation of the results previously reported by Missero et al. The p60 protein has been identified as a human cyclin A protein (37); however, one or both of the other two unidentified proteins, which are negatively regulated by E1A oncoproteins, may be identical to the putative novel transcription factors responsible for activating transcription of TGF-β RII.

E1A-mediated effects in other cell systems have also been demonstrated to occur through transcriptional down-regulation of specific gene expression. Transfection of mouse C2 myocytes with the E1A oncoprotein inhibits myogenic differentiation of these cells (37). E1A exerts its effect in these cells through two separate mechanisms: inhibition of expression of a myogenic regulatory factor, MyoD, and repression of MyoD-activated transcription (38). Both effects require the presence of two separate conserved regions of the E1A oncogene. These regions are identical to the domains of E1A required for transformation of keratinocytes, supporting the likelihood that the transforming properties of E1A similarly involve transcriptional regulation.

Adenovirus-host cell interactions have also served as an informative model for studying a particular type of transcrip-

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3 D. H. Kim, S. G. Choi, J. H. Chang, K. H. Lee, and S.-J. Kim, unpublished results.

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tional regulation involving nucleotide-specific DNA methylation. This mechanism for repressing gene transcription occurs in mammalian DNA most commonly at deoxyadenosine residues within dinucleotide couplings known as CpG islands. One such CpG island is located in the inactive target sequence of TGF-β type II receptor PRE1, and multiple CpG islands can also be found immediately flanking the PRE2 target sequence. Insertion of adenovirus DNA has been shown to alter methylation patterns of adjacent host DNA and, consequently, has clear potential to affect transcriptional activity of nearby promoter elements (39). Although DNA methylation is unlikely to contribute to the TGF-β type II receptor PRE1 and/or PRE2 sequences plays a complementary role in repressing TGF-β RII expression as well as viral oncogenesis, and we are currently performing a preliminary analysis of TGF-β RII promoter methylation patterns.

In summary, this study demonstrates that E1A transformation of keratinocytes is associated with down-regulation of TGF-β type II receptor expression resulting from a specific decrease in transcriptional activation of the TGF-β RII promoter region. The mechanism underlying this decreased expression has been shown to involve decreased interaction between several putative novel transcription factor proteins and their specific target sequences within the two positive regulatory regions, PRE1 and PRE2, of the TGF-β RII promoter. Further research is being performed to determine whether decreased binding is a consequence of decreased expression of the transcriptional factors, decreased activation of an inactive form, or competitive binding. The findings from our study provide additional support for the concept that the TGF-β type II receptor subserves an important tumor suppressor function in mammalian cells. Inactivation of the receptor in E1A-transformed keratinocytes disables the earliest stage of the TGF-β signaling pathway and abolishes TGF-β-mediated growth inhibition, and insofar as this model reproduces key general molecular events of carcinogenesis, transcriptional down-regulation of TGF-β RII may play a corresponding role in development of the TGF-β-resistant, aggressive cell growth demonstrated by various human carcinomas.

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REFERENCES
1. Reiss, M., and Sartorelli, A. (1987) Cancer Res. 47, 6705–6709
2. Alexandrow, M., and Moses, H. (1995) Cancer Res. 55, 1452–1457
3. Moses, H., Yang, E., and Pietra, J. (1990) Cell 53, 245–247
4. Oberhammer, F., Pivelka, M., Sharma, S., Tiefenbacher, R., Purichio, A., Sehgal, W., and Schulte-Hermann, R. (1995) Proc. Natl. Acad. Sci. U. S. A. 89, 5498–5412
5. Laiho, M., Weis, F. M. P., Boyd, F. T., Ignatz, R. A., and Massagué, J. (1991) J. Biol. Chem. 266, 9108–9112
6. Laiho, M., Weis, F. M. P., and Massagué, J. (1990) J. Biol. Chem. 265, 18518–18524
7. Mostakas, A., Lin, H. Y., Henis, Y. I., Plamondon, J., O’Connor-McCourt, M. O., and Lodish, H. (1993) J. Biol. Chem. 268, 22215–22218
8. Wraza, J. L., Attisano, L., Carcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F., and Massagué, J. (1994) Cell 71, 1003–1014
9. Roberts, A. B., and Sporn, M. B. (1990) PpEido Growth Factors and Their Receptors. Handbook of Experimental Pharmacology, pp. 419–472 Springer-Verlag, Heidelberg
10. Kimchi, A., Wang, X.-F., Weinberg, R., Cheifetz, S., and Massagué, J. (1988) Science 240, 196–199
11. Manning, A., Williams, A., Game, S., and Paraskava, C. (1991) Oncogene 6, 1471–1476
12. Sporn, M. B., and Roberts, A. B. (1985) Nature 313, 747–749
13. Park, K., Kim, S.-J., Bang, Y.-J., Park, J.-H., Kim, N. K., Roberts, A. B., and Park, K. (1993) Proc. Natl. Acad. Sci. U. S. A. 91, 8772–8776
14. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L. Z., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Brattain, M. G., and Willson, J. K. V. (1995) Science 269, 1336–1338
15. Myeroff, L. L., Parsons, R., Kim, S.-J., Hedrick, L., Cho, K. R., Orth, K., Mathis, M., Kinzler, K. W., Lutterbaugh, J., Park, K., Bang, Y.-J., Lee, H. Y., Park, J.-G., Lynch, H. T., Roberts, A. B., Vogelstein, B., and Markowitz, S. D. (1995) Cancer Res. 55, 5545–5547
16. Markowitz, S., and Roberts, A. B. (1996) Cytokine & Growth Factor Reviews, pp. 93–102, Vol. 7, Elsevier Science, Cambridge
17. Kim, D. H., and Kim, S.-J. (1996) J. Biol. Chem. 271, 143–158
18. Bae, H. W., Geiser, A. G., Sporn, M. B., Turner, M. T., Burmester, J. K., Sporn, M. B., Roberts, A. B., and Kim, S.-J. (1995) J. Biol. Chem. 270, 29469–29464
19. Trentin, J. J., Yabe, Y., and Taylor, G. (1962) Science 137, 835–841
20. Mayol, X., Graña, X., Baldi, A., Sang, N., Hu, Q., and Giordano, A. (1993) Oncogene 6, 481–485
21. Giordano, A., Whyte, P., Harlow, E., Franza, R., Jr., Beach, D., Draetta, G. (1989) Cell 56, 981–990
22. Giordano, A., McCall, C., Whyte, P., and Franza, R., Jr. (1991) Oncogene 6, 481–485
23. Whyte, P., Williamson, N. M., and Harlow, E. (1989) Cell 56, 67–75
24. Whyte, P., Ruley, H. E., and Harlow, E. (1989) J. Virol. 62, 257–265
25. Park, K., Bae, H., Heydemann, A., Roberts, A. B., Dotto, G. P., Sporn, M. B., and Kim, S.-J. (1994) Cancer Res. 54, 6087–6089
26. Akusjarvi, G., Kipling, E., and Roberts, J. R. (1986) Dev. Mol. Biol. 8, 53–95
27. Missero, C., Filvaroff, E., and Dotto, G. P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3489–3493
28. Kim, S.-J., Glick, A., Sporn, M. B., and Roberts, A. B. (1989) J. Biol. Chem. 264, 402–408
29. Kim, S.-J., Park, K., Rudkin, B. B., Dey, B. R., Sporn, M. B., and Roberts, A. B. (1994) J. Biol. Chem. 269, 3739–3744
30. Taipale, J., Matikainen, S., Hurme, M., and Keski-Oja, J. (1994) Cell Growth & Differentiation 5, 1309–1319
31. Ingalls, M., Mostakas, A., Lin, H. Y., Lodish, H. F., and Curr, B. I. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5359–5363
32. Sun, L., Wu, G., Willson, J. K. V., Zborowska, E., Yang, J., Rajkarunanayake, I., Wang, J., Gentry, L. E., Wang, X.-F., and Brattain, M. G. (1994) J. Biol. Chem. 269, 26449–26455
33. Wu, S., Theodorouscu, D., Kerbel, R., Willson, J. K. V., Mulder, K., Humphrey, L., and Brattain, M. (1992) J. Cell Biol. 116, 187–196
34. Birchennall-Roberts, M., Rossetti, F., Kasper, J., Lee, H. D., Friedman, R., Geiser, A., Sporn, M. B., Roberts, A. B., and Kim, S.-J. (1990) Mol. Cell. Biol. 10, 4978–4983
35. Okamoto, A., Jiang, W., Kim, S.-J., Spillare, E. A., Stoner, G. D., Weinstein, I. B., and Harris, C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11576–11580
36. Pines, J., and Hunter, T. (1990) Nature 346, 760–763
37. Webster, K. A., Muscat, G. E., and Kedes, L. (1988) Nature 332, 553–557
38. Caruso, M., Martelli, F., Giordano, A., and Felseni, A. (1993) Oncogene 8, 267–278
39. Doerfler, W. (1991) Adv. Virus Res. 39, 89
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