Estrogen Regulates the Association of Intermediate Filament Proteins with Nuclear DNA in Human Breast Cancer Cells*

(Received for publication, April 21, 1998, and in revised form, July 17, 1998)

Virginia A. Spencer, Amanda S. Coutts‡, Shanti K. Samuel, Leigh C. Murphy§, and James R. Davie¶

From the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E OW3, Canada

In a previous study we showed that the levels of the intermediate filament proteins, cytokeratins 8, 18, and 19, in the nuclear matrix-intermediate filament (NM-IF) fraction from the hormone-dependent and estrogen receptor (ER)-positive human breast cancer cell line T-47D5 were regulated by estrogens. In contrast, estrogens did not regulate the cytokeratins in the NM-IF fraction of the hormone-independent and ER-positive cell line, T5-PRF. In this study, human breast cancer cells were treated with cis-diaminedichloroplatinum to cross-link protein to nuclear DNA in situ, and proteins bound to DNA were isolated. We show that cytokeratins 8, 18, and 19 of T-47D5 and T5-PRF were associated with nuclear DNA in situ. The levels of the cytokeratins 8, 18, and 19 bound to nuclear DNA or associated with the cytoskeleton of T-47D5 human breast cancer cells decreased when estrogens were depleted or the pure antiestrogen ICI 164,384 was added. In contrast, the cytokeratin levels associated with nuclear DNA or cytoskeleton were not significantly affected by estrogen withdrawal or antiestrogen administration in T5-PRF cells. These observations suggest that estrogen regulates the organization of nuclear DNA by rearrangement of the cytokeratin filament network in hormone-dependent, ER-positive human breast cancer cells and that this regulation is lost in hormone-independent, ER-positive breast cancer cells.

The nuclear matrix (NM),¹ cytoskeleton (CSK), and extracellular matrix form the tissue matrix system, a mechanically continuous skeletal network thought to govern nuclear shape and function (1–3). The NM consists of a nuclear pore-lamina complex, residual nucleoli, and internal matrix (4). The NM is an RNA-protein structure that has a role in the organization and function of nuclear DNA (5, 6). The chromatin fiber is organized into loop domains by the association of DNA sequences, called matrix-attachment regions (MARs), located at the base of the loop with NM proteins (4, 7). Transcribing chromatin is also attached to the nuclear matrix by multiple dynamic attachment sites thought to be mediated by NM-bound transcription factors and histone-modifying enzymes (8–11).

In eukaryotic cells, the CSK is composed of actin-containing microfilaments, tubulin-containing microtubules, and intermediate filaments, which may be composed of keratins and vimentin (12). The NM is physically associated with the CSK through its associations with intermediate filaments, major proteins of the CSK (5, 13–15). In epithelial cells and in well and poorly differentiated carcinoma cells, cytokeratins 8, 18, and 19 are the major proteins of intermediate filaments (16).

Intermediate filaments are dynamic structures that may transmit signals from the extracellular matrix to the nucleus (13, 14, 16, 17). In an analysis of the NM-IF (nuclear matrix with associated intermediate filaments) fraction of T-47D5 human breast cancer cells (ER-positive and hormone-dependent), we identified the principal NM-IF proteins as cytokeratins 8, 18, and 19 (18). Estrogens regulated the levels of these cytokeratins in the NM-IF fraction of T-47D5 cells. When cells were grown in estrogen-depleted conditions, cytokeratin levels in NM-IF declined, but when estrogen was added back to the media, cytokeratin levels increased. In a hormone-independent, ER-positive human breast cancer cell line (T5-PRF), cytokeratins in the NM-IF were maintained at high levels regardless of whether the cells were grown in the presence or absence of estrogens (18). Furthermore, the pure antiestrogen, ICI 164,384, significantly reduced the NM-IF levels of cytokeratins 8, 18, and 19 in T-47D5 cells grown in estrogen-replete conditions, while having no effect on NM-IF levels of these proteins in the T5-PRF cell line.

There is both in situ and in vitro evidence that intermediate filaments bind to nuclear DNA (19–21). In vitro binding studies show that the intermediate filament protein vimentin selectively binds DNA sequences that are MARs, that are recognized by transcription factors, or that have structural properties important in recombination and gene expression (19).

In the identification of NM proteins bound to nuclear DNA in situ, the cross-linker cis-diaminedichloroplatinum (cis-DDP) has become particularly useful (11, 22, 23). Incubation of cells or nuclei with cis-DDP results in the cross-linking of protein to DNA in situ. Most proteins cross-linked to DNA with cis-DDP are nuclear matrix proteins, and the DNA cross-linked to protein is enriched in MAR sequences (21, 22, 24–27). Laminins, components of the nuclear pore-lamina, are cross-linked in situ to nuclear DNA consistent with in vitro data suggesting that these proteins are involved in the organization of nuclear DNA (26, 27).
In this study, we incubated human breast cancer cell lines, T-47D5 and T5-PRF, with cis-DDP to find if cytokeratins were bound to nuclear DNA in situ. We demonstrate that cytokeratins 8, 18, and 19 are attached to nuclear DNA in T-47D5 and T5-PRF cells. Further, we show that the interaction between cytokeratins and nuclear DNA is regulated by estrogens in hormone-dependent T-47D5 but not in the hormone-independent T5-PRF cell line. These results support the idea that intermediate filaments are involved in the organization of nuclear DNA.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The human breast carcinoma cell lines T-47D5 (28) and T5-PRF (18) were used. All cell lines were maintained at 37 °C (humidified atmosphere, 5% CO2/95% air) on 150 × 20-mm tissue culture dishes (Nunc) in culture medium containing Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Inc.) supplemented with 1% (v/v) l-glutamine (stock solution concentration, 200 mM), 1% (v/v) glucose (stock solution concentration, 30% v/v), 1% (v/v) penicillin/streptomycin (stock solution concentrations, 10,000 units/ml and 10,000 μg/ml, respectively), and 5% (v/v) fetal calf serum (FCS, Life Technologies, Inc.) except for T5-PRF, which were maintained in medium containing Dulbecco’s modified Eagle’s medium-phenol red free (Sigma), 5% (v/v) twice charcoal-stripped FCS and supplemented as mentioned above (5% CS) (18). T-47D5 cells acutely depleted of estrogen were grown in 5% CS for one passage, whereas T5-PRF cells were routinely passaged in 5% CS. Cells were passaged at 70–80% confluency using Earle’s EDTA solution and set up in 100-mm dishes for experiments. For estrogen and antiestrogen treatments, the T-47D5 or T5-PRF cells were cultured for 72 h in the presence of 10 nM estradiol with or without 1 μM ICI 164,384 or the ethanol vehicle. Following this incubation, cells were treated with lysis buffer (5M urea, with or without 1M thiourea instead of 5M urea). The proteins were released from the hydroxylapatite resin and were cross-linked to DNA. To reverse the cross-linking, the hydroxylapatite resin was incubated in lysis buffer that had 1M thiourea instead of 5M urea. The proteins were released from the hydroxylapatite, whereas the DNA remained bound. The released proteins were isolated according to methodologies previously reported (29).

**Cytokeratin Extraction**—Cytoskeletal protein-enriched fractions were isolated according to the method of Sommers and colleagues (30). Cells were suspended in 4 °C Triton-high salt buffer (20 mM Tris-HCl, pH 7.4, 0.6 mM KCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride), incubated on ice for 20 min, and then centrifuged at 10,000 × g for 20 min. The pellet was then resuspended in the same Triton-high salt buffer incubated on ice for 20 min, and then centrifuged once more. The pellet of insoluble proteins was resuspended in 8 μl urea.

**Isolation of Nuclear Matrix Proteins**—Nuclear matrices were prepared according to methodologies previously reported (29).

**RESULTS**

**Levels of cis-DDP DNA-Cross-linked Cytokeratins 8, 18, and 19 Are Influenced by Estrogen and Antiestrogen in a Hormone-dependent Human Breast Cancer Cell Line**—Our previous study showed that the levels of cytokeratins in the NM-IF fraction of T-47D5 cells were regulated by estradiol (18). To determine whether the interaction between cytokeratins and nuclear DNA was regulated by estradiol, the effect of estradiol withdrawal and re-addition on cytokeratin-DNA interaction was investigated. T-47D5 cells grown in the absence (one passage) or presence of estradiol were incubated with cis-DDP, and the proteins cross-linked to DNA were isolated. The cross-linked proteins were electrophoretically resolved on two-dimensional gels (Fig. 1) and one-dimensional SDS-polyacrylamide gels (Fig. 2A). A comparison of Fig. 1B with Fig. 1A shows that when cells were grown in the absence of estradiol, there was a reduction in the relative amounts of cytokeratins 8, 18, and 19 bound to nuclear DNA. The addition of estradiol to cells grown in the absence of estradiol for one passage had a dramatic effect on the amount of cytokeratins binding to nuclear DNA in situ, with the levels of cytokeratins 8, 18, and 19 showing marked increases in abundance (Fig. 1C). Inspection of the two-dimensional gel patterns showed that cytokeratins 8 and 19 did not co-migrate with other proteins with an identical molecular weight. This situation did not apply to cytokeratin 18, which co-migrated with other proteins. However, the two-dimensional gel showed that the levels of cytokeratin 18 followed a similar trend to levels displayed in the one-dimensional gel. The cytokeratins were not the only DNA-binding proteins whose levels were affected when cells were grown in the presence or absence of estrogen. However, not all cis-DDP cross-linked DNA-binding proteins were responsive to estrogen; for example, the levels of nuclear matrix proteins NMP-5, NMP-6, and hnRNP K were similar in the three preparations.

The proteins cross-linked to nuclear DNA of cells incubated with or without estradiol were resolved on SDS-polyacrylamide gels (Fig. 2A), and the relative levels of the cytokeratins were determined by densitometric scanning of the Coomassie Blue-stained gels (see “Experimental Procedures”). The abundance of DNA-bound cytokeratins 8, 18, and 19 in cells grown in the absence of estrogen was reduced to levels of 0.60 ± 0.08, 0.64 ± 0.02, and 0.59 ± 0.11 (n = 3), respectively (Fig. 2B), of the cells grown in the presence of estrogen. When estrogen was added back to the media of the cells cultured in the absence of estrogen, the levels of the DNA-attached cytokeratins 8, 18, and 19 rebounded to levels higher than those found in T-47D5 cells cultured in the presence of estrogens (1.95 ± 0.22, 1.59 ± 0.22, and 1.61 ± 0.17, n = 3, respectively) (Fig. 2B). The proteins shown in Figs. 1 and 2 were from cells cross-linked with 3 and 1 μM cis-DDP, respectively. The relative levels of DNA-bound cytokeratin in cells grown with and without estrogens and

---

**Estrogen Regulation of Cytokeratin-DNA Interactions**

---

29094
treated with 3 mM cis-DDP yielded similar results to those treated with 1 mM cis-DDP.

Treatment of T-47D5 cells with the antiestrogen ICI 164,384 reduced the amount of cytokeratins associated with the NM-IF fraction (18). The addition of ICI 164,384 had the same effect on the amount of cytokeratin interacting with nuclear DNA, with the amount of DNA-cross-linked cytokeratins 8, 18, and 19 being reduced to low levels (Fig. 2C).

Levels of cis-DDP DNA-Cross-linked Cytokeratins 8, 18, and 19 Are Not Influenced by Estrogen or Antiestrogen in an ER-positive, Hormone-independent Human Breast Cancer Cell Line—The growth of the T5-PRF cell line (ER positive) is nonresponsive to estrogen, and its sensitivity to ICI 164,384 is less than that of T-47D5 cells (18). The NM-IF preparations from these cells had high amounts of cytokeratins 8, 18, and 19, regardless of whether the cells were grown in the presence or absence of estradiol (18). To determine whether the interaction between cytokeratins and DNA was nonresponsive to estrogen, proteins cross-linked to nuclear DNA in T5-PRF cells grown in the presence or absence of estrogen were isolated and analyzed by gel electrophoresis. The levels of DNA-bound cytokeratins 8, 18, and 19 in T5-PRF cells grown in the absence of estrogens were greater than those of DNA-bound cytokeratins of T-47D5 cells grown with estrogen (2.87 ± 0.9, 2.0 ± 0.41, 1.93 ± 0.16, n = 3, respectively) (Fig. 3, A and B). Incubation of T5-PRF cells with estrogen or ICI 164,384 in the presence of estrogen did not significantly alter the levels of cis-DDP DNA-cross-linked cytokeratins 8, 18, and 19 (Fig. 3).

Estrogen Affects the Cellular Levels of Cytokeratins in T-47D5 but Not T5-PRF Human Breast Cancer Cells—To determine whether estrogen affected the cellular levels of cytokeratins in hormone-responsive breast cancer cells, a high-salt Triton-insoluble cellular fraction, which contains 95% of the total cellular cytokeratins (32), was isolated from T-47D5 and T5-PRF cells grown in the presence or absence of estrogen. Fig. 4 shows the two-dimensional gel patterns of the cytoskeletal protein-enriched fractions. This fraction from T-47D5 cells grown in the presence of estradiol had a much greater level of cytokeratins 8, 18, and (to a lesser extent) 19 than that from cells grown in the absence of estradiol. Treatment of T-47D5 cells with the antiestrogen ICI 164,384 in the presence of estrogen resulted in a major reduction in total cellular levels of estrogen.
cytokeratins (data not shown). In contrast, the levels of cytokeratins in the cytoskeletal protein-enriched fractions from T5-PRF cells grown with or without estradiol (and ICI 164,384; data not shown) were similar (Fig. 4). The levels of other major proteins in the T-47D5 or T5-PRF preparations were not affected in cells grown in the presence or absence of estradiol. Thus, the effect of estradiol in T-47D5 cells did not globally affect other cytoskeletal proteins.

DISCUSSION

Nuclear matrix proteins have a key role in the organization of nuclear DNA into loop domains. Several MAR-binding proteins have been identified by in vitro methods, but few have been shown to bind to nuclear DNA in situ. Recently, Göhring and Fackelmayer (33) presented evidence that SAF-A, a MAR-binding protein, was associated with DNA in situ. The cross-linking agent used was formaldehyde, which can cross-link protein to DNA. However, formaldehyde will also cross-link protein to protein; thus, indirect cross-linking between protein and DNA may occur (34). cis-DDP cross-links DNA to protein (cross-link distance, 4 Å) (21). This cross-linker preferentially cross-links nuclear matrix proteins to MAR DNA, making this reagent particularly useful in the identification of nuclear matrix proteins involved in the organization of nuclear DNA (11).

Our results extend the original observations of Hnilica and colleagues (21, 35), who reported that cis-DDP cross-linked cytokeratins to nuclear DNA in Novikoff hepatoma cells. Cytokeratins 8, 18, and 19 are among the most prominent proteins cross-linked to DNA in the MDA MB 231 cells reflect the lower expression of these cytoskeletal proteins in this cell line (36, 37). Although cytokeratins lack a nuclear localization signal, they have been shown to bind to nuclear DNA in situ (5, 14). Intermediate filaments are thought to penetrate the double nuclear membrane and are part of the nuclear peripher-

Fig. 3. Effect of estrogen and antiestrogens on levels of cytokeratins cross-linked to DNA by cis-DDP in T5-PRF human breast cancer cells. A and B, cells were cross-linked with 1 mM cis-DDP. Ten μg of protein cross-linked to DNA was electrophoretically resolved on a SDS-polyacrylamide gel. The gels were stained with Serva Blue. A: lane 1, T5-PRF cells cultured in the absence of estrogen (5% CS); lane 2, T5-PRF cells cultured in the presence of 10 nM estrogen for 72 h. B: lane 1, T5-PRF cells cultured in the presence of estrogen (5% CS) for 72 h; lane 2, T5-PRF cells cultured in the presence of estrogen (10 nM) and ICI 164,384 (1 μM) for 72 h. The position of the molecular weight standards (in thousands) is shown on the left side of the gel. C: T5-PRF cells cultured in the absence of estrogen (5% CS) were cross-linked with 3 mM cis-DDP, and 20 μg of protein cross-linked to DNA was electrophoretically resolved on a two-dimensional gel. The gel was stained with silver. K8, K18, and K19 identify the cytokeratins 8, 18, and 19, respectively. Two nuclear matrix proteins used as internal standards are shown as NMP5 and NMP6. hK designates hnRNP K.

Fig. 4. Effect of estrogens on cytokeratin levels in T-47D5 and T5-PRF human breast cancer cells. High-salt Triton-insoluble proteins were isolated from T-47D5 and T5-PRF human breast cancer cells cultured in estrogen-replete and estrogen-depleted conditions. The proteins (15 μg) were electrophoretically resolved on two-dimensional gels. The gels were stained with silver. A, T-47D5 cells grown without estrogens for one passage (5% CS); B, T-47D5 cells grown in the absence of estrogen for one passage and then cultured in the presence of estrogen (10 nM) for 72 h; C, T-5-PRF cells cultured in the absence of estrogen (5% CS); D, T5-PRF cells continuously grown in the absence of estrogen were cultured in the presence of estrogen (10 nM) for 72 h. K8, K18, and K19 identify the cytokeratins 8, 18, and 19, respectively.
Estrogen Regulates the Cytokeratin-DNA Interactions

Estrogen regulates the cytoskeletal levels of cytokeratins and the interaction between cytokeratins and nuclear DNA in ER-positive, hormone-dependent breast cancer cells. The removal of estrogen or the introduction of an estrogen antagonist (ICI 164,384) results in decreased levels of intermediate filaments composed of cytokeratins and a corresponding decline in cytokeratin associations with the nuclear matrix and binding to nuclear DNA (18) (this study). Rearrangement of the keratin intermediate filaments (called tonofilaments) in MCF-7 (ER-positive, hormone-dependent) human breast cancer cells was observed when cells were deprived of estrogens or treated with antiestrogens (39). Further, in agreement with our observations, the latter study observed that administration of estrogen increased the keratin filamentous network in the breast cancer cells. It is also expected that a reorganization of chromatin will accompany this rearrangement of the tonofilaments. In contrast, an ER-positive human breast cancer cell line, T5-PRF, that has acquired the ability to grow normally in a medium greatly depleted of estrogens had higher amounts of cytokeratins associated with the cytoskeleton, nuclear matrix, and DNA than did the parent cell line cultured with estrogen. Regardless of the presence or absence of estradiol or the administration of an antiestrogen, the cytokeratin interaction with nuclear DNA was not affected. Thus, the T5-PRF cell line has lost the capacity of estrogen to regulate cytokeratin interaction with nuclear DNA. The organization of nuclear DNA mediated by cytokeratins will be maintained in these cells regardless of whether estrogen is present or absent. These observations are particularly important in breast cancer, as progression of breast epithelial cells to malignancy is accompanied by increased expression of cytokeratins 8 and 18 (36).

Nuclear matrix proteins are informative biomarkers in analysis of many types of cancer (40). During the preparation of nuclear matrix proteins, intermediate filaments are often removed (29). Our study and those of Hnilica and colleagues (21, 27) show that cytokeratins are nuclear matrix proteins associated with nuclear DNA. These observations argue that informative nuclear matrix proteins may be lost during previously used protocols for the preparation of nuclear matrix proteins. Thus, the characterization of nuclear matrix proteins and proteins cross-linked to DNA by cis-DDP in situ, which are primarily nuclear matrix proteins, are two complementary approaches that can be used to identify nuclear matrix proteins that are informative in cancer diagnosis.

REFERENCES

1. Maniotis, A. J., Chen, C. S., and Ingber, D. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 849–854
2. Bourgeau, N., Myers, C. V., and Bissell, M. J. (1995) Trends Cell Biol. 5, 1–4
3. Bidwell, J. P., Alvarez, M., Feister, H., Onyia, J., and Hock, J. (1998) J. Bone Miner. Res. 13, 155–167
4. Davie, J. R. (1995) Int. Rev. Cytol. 162A, 191–250
5. Nickerson, J. A., Krockmalnic, G., He, D. C., and Penman, S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2259–2263
6. Jackson, D. A., and Cook, P. R. (1986) EMBO J. 7, 3667–3677
7. Bode, J., Schlaik, T., Rios-Ramirez, M., Mielke, C., Stengert, M., Kay, V., and Kiehl-Wurth, D. (1995) Int. Rev. Cytol. 162A, 389–454
8. McNiel, S., Guo, B., Stein, J. B., Lian, J. B., Budhneyer, S., Seto, E., Atchison, M. G., Penman, S., van Wijnen, A. J., and Stein, G. S. (1998) J. Cell. Biochem. 68, 500–510
9. Zeng, C., McNiel, S., Pockwinse, S., Nickerson, J., Shapland, L., Lawrence, J. B., Penman, S., Heiber, S., Lian, J. B., van Wijnen, A. J., Stein, J. L., and Stein, G. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1585–1589
10. Davie, J. R. (1997) Mol. Biol. Rep. 24, 197–207
11. Davie, J. R., Samuel, S., Spenceer, V., Bajino, L., Sun, J.-M., Chen, H. Y., and Holth, L. T. (1998) Gene Ther. Mol. Biol. 1, 509–526
12. Inger, B. (1993) J. Cell Sci. 104, 613–627
13. Hendrix, M. C. J. (1996) Cancer Metastasis Rev. 15, 413–416
14. Penman, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5251–5257
15. Georgatos, S. D., and Maison, C. (1996) Int. Rev. Cytol. 164, 91–138
16. Oshima, R. G., Baribault, H., and Cailin, C. (1996) Cancer Metastasis Rev. 15, 445–471
17. Klymkowsky, M. W. (1996) Cancer Metastasis Rev. 15, 417–428
18. Coutts, A. S., Davie, J. R., Dotzlaw, H., and Murphy, L. C. (1996) J. Cell. Biochem. 63, 174–184
19. Wang, X., Tolstenson, G., Shoeman, R. L., and Traub, P. (1996) DNA Cell Biol. 15, 209–225
20. Ward, W. S., Schmidt, W. N., Schmidt, C. A., and Hnilica, L. S. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 419–423
21. Olinski, R., Wedrychowski, A., Schmidt, W. N., Briggs, R. C., and Hnilica, L. S. (1987) Cancer Res. 47, 201–205
22. Ferraro, A., Eufemi, M., Cervoni, L., Altieri, F., and Turano, C. (1995) Acta Biochim. Pol. 42, 145–152
23. Mattia, E., Eufemi, M., Chichiarelli, S., Ceridono, M., and Ferraro, A. (1995) Exp. Cell Res. 228, 216–219
24. Ferraro, A., Grandi, P., Eufemi, M., Altieri, F., and Turano, C. (1992) FEBS Lett. 307, 383–385
25. Bubley, G. J., Xu, J., Kupiec, N., Sanders, D., Foss, F., O’Brian, M., Emi, Y., Teicher, B. A., and Patierno, S. R. (1996) Biochem. Pharmacol. 51, 717–721
26. Wedrychowski, A., Bhorje, J. S., and Briggs, R. C. (1989) Exp. Cell Res. 183, 376–387
27. Wedrychowski, A., Schmidt, W. N., and Hnilica, L. S. (1986) J. Biol. Chem. 261, 3570–3576
28. Watts, C. K., Handel, M. L., King, R. J., and Sutherland, R. L. (1992) J. Steroid Biochem. Mol. Biol. 41, 529–536
29. Samuel, S. K., Minsh, T. M., and Davie, J. R. (1997) Cancer Res. 57, 147–151
30. Sommers, C. L., Walker-Jones, D., Heckford, S. E., Worland, P., Valverius, E. C., Clark, R., McCormick, F., Stampfer, M., Ablarach, S., and Gelmann, E. P. (1989) Cancer Res. 49, 4258–4263
31. Ferraro, A., Grandi, P., Eufemi, M., Altieri, F., Cervoni, L., and Turano, C. (1991) Biochem. Biophys. Res. Commun. 178, 1365–1370
32. Chou, C. F., Roppel, C. L., Rott, L. S., and Omary, M. B. (1993) J. Cell. Sci. 103, 433–444
33. Göhring, F., and Facklemeyer, F. O. (1997) Biochemistry 36, 8267–8283
34. Walter, J., and Biggin, M. D. (1997) Methods 11, 215–224
35. Wedrychowski, A., Schmidt, W. N., Ward, W. S., and Hnilica, L. S. (1986) Biochemistry 25, 1–9
36. Trask, D. K., Band, V., Zajchowski, D. A., Yaswen, P., Suh, T., and Sager, R. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2319–2323
37. Kuo, J. W., Thompson, D., Hock, R. V., and Weigel, R. J. (1998) Nucleic Acids Res. 26, 1116–1123
38. Khan, M. U., and Sadler, P. J. (1978) Chem. Biol. Interact. 21, 227–232
39. Saponi, A., Pietri, P., Bussolati, G., and Marchisio, P. C. (1986) Cancer Res. 46, 2550–2551
40. Reploge-Schwab, T. S., Pienta, K. J., and Getzenberg, R. H. (1996) Crit. Rev. Eukaryotic Gene Expression 6, 103–113