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Endogenous Retroviruses and Breast Carcinoma Development DAMD17-94-J-4059

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Actively synthesizing endogenous retroviral DNA, such as the intracisternal A-particle (IAP) DNA, has been shown to transpose in murine tumor cells and act as an insertional mutagen causing aberrant expression of target genes and contributing to neoplastic transformation. Expression of a human endogenous retrovirus HERV-K10, which is closely related to the IAP gene, has been observed in normal lymphocytes and leukemic cells, in teratocarcinoma cells, and in a breast cancer cell line T47D. Expression in T47D cells is steroid hormone-dependent, being activated by estrogen and progesterone. As a step to understanding the role of HERV in breast tumorigenesis, we investigated HERV-K10 expression in the MCF-7 and BT-20 breast carcinoma cell lines, which are estrogen/progesterone receptor positive and negative, respectively. We have cultured the two cell lines in the presence and absence of estrogen plus progesterone and isolated the RNA. Using primers located within the long terminal repeat of HERV-K10, we investigated the expression of HERV-K10 by reverse transcriptase and polymerase chain reaction (RT-PCR). We have shown that HERV-K10 is expressed in both cell types, and expression is hormone independent. The results suggest that HERV-K10 may be involved in DNA mutagenesis in breast tumors.

Human Endogenous Retrovirus; Expression; RT-PCR; Breast Tumor Cells 8

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Chris Hamm 9-25-95

PI - Signature Date

Endogenous Retroviruses and Breast Cancer Development

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INTRODUCTION

While most endogenous retroviruses are transcriptionally silent and considered nonpathogenic (1), there is growing evidence that the expression of certain endogenous retroviruses is etiologically involved in some aspects of multistage carinogenesis. A wide variety of spontaneous and chemically-induced tumors in rodents exhibit elevated levels of endogenous retrovirus sequences, including those belonging to the intracisternal A-particle (IAP)-type, C-type, mouse mammary tumor virus (MMTV)-type and virus-like (VL30) sequences (2-6). The murine IAP proviral sequence are of particular interest because of recent indication that its expression is associated with breast cancer. IAPs are rarely found in most normal adult cells, but they are expressed in early embryogenesis, the normal thymus of young mice, and many different types of tumors and leukemias (5,6). In murine mammary carcinomas, the IAP gene is expressed in mouse hyperplastic alveolar nodules induced by hormonal, chemical, or viral stimuli and in primary adenocarcinomas arising from the nodules (5,7).

In tumor cells where IAP expression is active, IAP elements transpose and act as insertional mutagenes of DNA via integration of actively synthesizing extrachromosomal viral DNA to new sites in the cell genome. Such transpositions cause aberrant expression of target genes such as oncogenes and genes encoding growth factors, growth-factor receptors, cytokines, and cytokine receptors. Some of these alterations result in augmented growth autonomy of the host cell, and directly contribute to neoplastic transformation (5,6,8).

Expression and association of human endogenous retroviruses (HERV) with diseases is not yet well explored. A human virus, HERV-K10, closely related to the IAP gene, has been cloned and shown to contain a pol region closely related to type A (IAP of rodent), type D and type B (MMTV) retroviruses (9). Expression of HERV-K10 thus far, have been observed in normal lymphocytes and leukemic cells, in teratocarcinoma cells, and in a breast cancer cell line T47D (10-13). Expression in T47D cells is steroid hormone dependent, being activated by estrogen and progesterone (14). Prognosis of human breast cancers is associated with their ability to respond to hormone treatment. Those responding to estrogen analog Tomoxifen treatment will have a lower risk of relapse and better overall survival rate (15,16). As a step to understand the role of HERV in breast tumorigenesis, in the present study, we have investigated the expression, by reverse transcriptase-polymerase chain reaction (RT-PCR), of HERV-K10 in MCF-7, and BT-20 breast carcinoma cell lines, the former being estrogen and progesterone receptors positive while the latter estrogen and progesterone receptor negative. We have shown that HERV-K10 is expressed in both cell lines in a hormone-independent manner.

BODY OF WORK

To analyze the expression of HERV-K10 in MCF-7 and BT-20 breast carcinoma cell lines and to investigate whether the expression is hormone dependent, log phase cells were cultured in phenol red-free DME containing 5% dextran-coated charcoal-stripped fetal calf serum for >24 hr to eliminate the effects of endogenous hormones. The medium was then changed to contain 10 nM estradiol followed by 100 nM progesterone for an additional 24 hr each. As controls, cells were also cultured in the stripped medium without hormones. RNA were isolated from cells as described (17) and used for RT-PCR. The RT reaction was performed according to the manufacture's instructions (BRL). Amplification of the resulting cDNA was accomplished by the PCR using primers located within the long terminal repeat of HERV-K10 at nt 246-260 and nt 510-528. The resulting 291 bp PCR products (including an *Eco*RI site on the reverse primer) were analyzed by electrophoresis in an 1.4% agarose gel. HeLa cells, which are known to express HERV-K10 (14), were used as a positive control. As a control for the RT reaction, PCR to elongation factor-2 (EF-2) was carried out using primers designed to yield a 242 bp product. Figure 1 shows that all three cell lines yielded PCR products of the expected size for both HERV-K10 and EF-2, indicating that both breast cancer cell lines express HERV-K10. The expression is independent of hormone treatment since cells treated with or without steroid hormones gave positive results. PCRs carried out on samples prepared in the absence of reverse transcriptase did not yield detectable products, suggesting that DNA contamination in the RNA samples was negligible.

CONCLUSIONS

We have shown that HERV-K10 is expressed in MCF-7 and BT-20 human breast carcinoma cells and that the expression is not steroid hormone-dependent. However, the expression in normal cells is yet to be examined to determine whether active HERV-K10 expression is associated with breast tumor cells but not with normal cells. Currently we are analyzing the normal human keratinocytes. The absence of HERV-K10 expression in keratinocytes would support our hypothesis that in breast tumors actively expressing HERV may be inducing transposition-mediated mutagenesis and thus tumorigenesis.

Ono showed that HERV-K10 expression in human T47D breast carcinoma cells is dependent on estrogen and progesterone treatment (14). However, we showed that expression in MCF-7 and BT-20 cells is not hormone dependent. Like T47D cells (18,19), MCF-7 cells contain receptors for estrogen and progesterone (20). Thus, our results were contrary to expectations. A simple explanation is that PCR is not quantitative enough to reveal the differences in expression between hormone treated and untreated cells. We intend to use RNase protection assays and Northern hybridizations, both being more quantitative, to investigate whether HERV-K10 expression is hormone dependent in the next fiscal year.

Although cultured cell lines has provided valuable leads in the study of mechanisms of tumor development and progression, they are known not to faithfully mimic physiology of tumors. Thus, we intend to expand our study to tumor tissues in the next fiscal year.

REFERENCES

1. Weiss, R., Teich, N., Varmus, H. and Coffin, J. eds. 1984. RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. Dragani, T.A., Manenti, G., Porta, G.D., Gattoni-Celli, S., and Weinstein, I.B. 1986. Expression of retroviral sequences and oncogenes in murine hepatocellular tumors. Cancer Res. 46:1915-1919.
3. Graham, D.E. Medina, D., and Smith, G.H. 1984. Increased concentration of an indigenous proviral mouse mammary tumor virus long terminal repeat-containing transcript is associated with neoplastic transformation of mammary epithelium in C3H/Sm mice. J. Virol. 49:819-827.
4. Kirschmeier, P., Gattoni-Celli, S., Dina, D., and Weinstein, I.B. 1982. Carcinogen- and radiation-transformed C3H 10T 1/2 cells contain RNAs homologous to the long terminal repeat sequence of a murine leukemia virus. Proc. Natl. Acad. Sci. USA 79:2773-2777.
5. Kuff, E.L. 1990. Intracisternal A particles in mouse neoplasia. Cancer Cells 2:398-400.
6. Kuff, E.L. and Lueders, K.K. 1988. The intracisternal A-particle gene family:structure and functional aspects. Adv. Cancer Res. 51:183-275.
7. Asch, B.B., Acsh, H.L., Stoler, D.L., and Anderson, G.R. 1993. De-regulation of endogenous retrotransposons in mouse mammary carcinomas of diverse etiologies. Int. J. Cancer 54:813-819.
8. Leslie, K.B., Lee, F., and Schrader, J.W. 1991. Intracisternal A-type particle mediated activation of cytokine genes in a murine myelomonocytic leukemia: generation of functional cytokine mRNAs by retroviral splicing events. Mol. Cell. Biol. 11:5562-5570.
9. Ono M. Yasunaga, T., Miyata, T., and Ushikubo, H. 1986. Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome. J. Virol. 60:589-598.
10. Medstrand, P., Lindeskog, M., and Blomberg, J. 1992. Expression of human endogenous retroviral sequences in peripheral blood mononuclear cells of healthy individuals. J. Gen. Virol. 73:2463-2466.
11. Brodsky, I., Foley, B., Haines, D., Johnston, J., Cuddy, K., and Gillespie, D. 1993. Expression of HERV-K proviruses in human leukocytes. Blood 81(9):2369-2374.
12. Lower, R., Lower, J., Tonder-Koch, C., and Kurth, R. 1993. A general method for the identification of transcribed retrovirus sequences (R-U5 PCR) reveals the expression of the human endogenous retrovirus loci HERV-H and HERV-K in teratocarcinoma cells. Virology 192:501-511.
13. Boller, K., Konig, H., Sauter, M., Mueller-Lantzsch, N., Lower, R., Lower, J., and Kurth, R. 1993. Evidence that HERV-K is the endogenous retrovirus sequence that codes for the human teratocarcinoma-derived retrovirus HTDV. Virology 196:349-353.
14. Ono, M., Kawakami, M., and Ushikubo, H. 1987. Stimulation of expression of the human endogenous retrovirus genome by female steroid hormones in human breast cancer cell line T47D. J. Virol. 61:2059-2062.
15. Fuqua, S.A.W., Chamness, G.C., and McGuire, W.L. 1993. Estrogen receptor mutations in breast cancer. J. Cell. Biochem. 51:135-139.
16. McGuire, W.L., Chamness, G.C., and Fuqua, S.A.W. 1991. Estrogen receptor variants in clinical breast cancer. Mol. Endocrinol. 5:1571-1577.
17. Chomczynski, P. and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162(1):156-159.
18. Chalbos, D., Vignon, F., Keydar, I., and Rochefort, H. 1982. Estrogens stimulate cell proliferation and induce secretory proteins in a human breast cancer cell line (T47D). J. Clin. Endocrinol. Metab. 55:276-283.
19. Keydar, I., Chen, L., Karbey, S., Weiss, F.R., Delarea, J., Radu, M, Chaitcik, S., and Brenner, H.J. 1979. Establishment and characterization of a cell line of human breast carcinoma origin. Eur. J. Cancer 15:659-670.
20. Fuqua, S.A.W., Hill, S.M., Chamness, G.C., Benedix, M.G., Greene, G.L., O'Malley, B.W., and McGuire, W.L. 1991. Progesterone receptor gene restriction fragment length polymorphisms in human breast tumors. J. Natl. Cancer Inst. 83:1157-1160.

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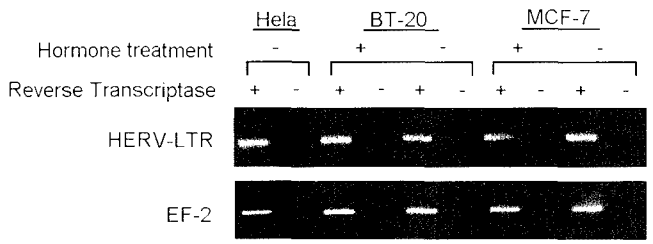

Figure 1. RT-PCR products. Cells were grown in charcoal-stripped, phenol red-free medium with (+) or without (-) the addition of estradiol plus progesterone. One microgram of total RNA was reverse transcribed using an oligo-dT primer. Control reactions without RT were done for each cell line. The cDNA was amplified by PCR using primers to the long terminal repeat of HERV-K10 designed to yield a 291 bp product or primers to the EF-2 gene designed to yield a 242 bp product.