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The changes in the gene program of neoplastic cells that regulate the expression of an invasive phenotype are largely undefined. Direct comparisons of the gene expression profile displayed in normal and carcinomatous breast tissues have provided insights into the mechanisms underlying tumor progression. However, attempts to identify the gene products differentially expressed during invasion *in vivo* have been hampered by the fact that only a small percentage of the cells recovered from a tumor mass are actively engaged in invasive behavior at the time of isolation. Because tissue remodeling induced during mammary gland involution bears homology to early stages of carcinogenesis, the involuting mammary gland may be used to identify genes that control matrix turnover in cancerous states. To this end, we propose to *i) generate cDNA libraries from control versus involuting mouse mammary glands, ii) isolate differentially expressed genes during matrix remodeling, iii) identify differentially expressed genes that encode secretory proteins associated with the involution program and iv) identify human homologues of the mouse-derived matrix remodeling genes.* The approach should allow for the identification of gene products relevant to breast cancer invasion.

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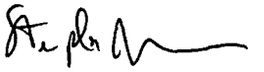

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I. INTRODUCTION

Current evidence suggests that breast carcinoma cells invade local tissues and metastasize by i) altering their cell-cell and cell-matrix interactions, ii) displaying an aberrant motile phenotype, and iii) either synthesizing, or inducing the synthesis of, proteolytic enzymes that degrade the structural barriers established by the extracellular matrix[1-3]. The complex changes in the gene program of neoplastic cells that regulate the expression of this phenotype are largely undefined, but increased interest has focused on identifying those genes that are specifically overexpressed in human breast cancer[(e.g., 3-10)]. Such information not only provides new insights into the cellular factors that control tissue-invasive behavior, but may also lead to improvements in patient diagnosis and to the more rational design of therapeutic interventions[3-10]. Consistent with this rationale, direct comparisons of the gene expression profile displayed in normal versus neoplastic breast cancer cell lines, or normal and carcinomatous breast tissues, have provided a number of novel insights into the mechanisms and processes underlying tumor progression[6-11]. Interestingly, despite the power of the analytical techniques employed for these purposes, the number of differentially expressed genes identified thus far are - at first glance - perplexingly small, despite the striking changes known to occur in cellular behavior[(e.g., 7,8)]. However, analyses of breast cancer cell lines grown *in vitro* or static tumor masses recovered from *in vivo* sites of disease may be problematic. First, comparisons between normal and neoplastic breast cancer cell lines grown atop plastic substrata *in vitro* will not recapitulate the complex interactions known to occur across the carcinoma-mesenchymal cell axis *in vivo*[1,2]. Indeed, many of the most interesting gene products that have been associated with the expression of tissue-invasive phenotypes in breast cancer tissue are synthesized by surrounding stromal cells rather than the tumor itself[2,3,10]. Secondly, while the gene expression patterns identified in tissues recovered from *in vivo* sites clearly circumvent the limitations inherent in the *in vitro* studies, only a small percentage of the cells recovered from a tumor mass at a single, fixed time point would be expected to be actively engaged in invasive behavior. Given the many similarities between developmental/tissue repair processes and malignant growth (re; the ability of cancer cells inappropriately recapitulate developmental programs associated with epithelial-mesenchymal cell transitions or repair programs associated with wound healing[12,13]), we have considered the possibility that the *in situ* induction of a synchronous matrix remodeling program in normal tissues would allow for the more efficient isolation of those gene products critical to cancer cell invasion. Indeed, recent studies have demonstrated that gene expression patterns associated with the tissue remodeling program induced during the involution of the normal lactating mammary gland bear considerable overlap with those detected in the early stages of carcinogenesis (e.g., stromeylsin-1, stromeylsin-3, urokinase-type plasminogen activator, tissue inhibitor of metalloproteinases[14-16]). Hence, we propose to use the involuting mammary gland explant model as a means to rapidly enrich for, and identify, the subset of genes that control the disassembly of the extracellular matrix in cancerous states. Furthermore, by selectively identifying the subset of gene products that encode secreted proteins in breast cancer tissue, new diagnostics as well as novel targets for therapeutic intervention can be rapidly identified.

II. BODY

To begin characterizing those gene products that are induced in the involuting mammary gland, differentially expressed genes are being isolated by a subtractive hybridization protocol

established in our laboratory. Because most subtractive hybridization schemes require prohibitive amounts of poly(A)$^+$ mRNA, we have opted to employ an alternative approach recently outlined by Lin et al wherein both driver (i.e., "control") and target (i.e., "test") cDNA libraries are constructed in phagemid vectors. Subsequently, one of the cDNA libraries is used to prepare single-stranded (ss) DNA, and the other to produce complementary biotinylated RNA by *in vitro* transcription. By subtracting lactating (wherein the expression/synthesis of matrix-degrading proteinases is minimal[15,16]) from involuting explants, panels of induced genes will be isolated. [We note that involuting cultures can likewise be subtracted from lactating explants in order to isolate repressed transcripts.]

To first prepare the relevant RNAs, lactating and involuting mammary gland explant cultures were prepared according to the technique originally described by Banerjee et al. as modified by Young[17]. In brief, 3-4 week-old BALB/C mice were injected subcutaneously for nine consecutive days with progesterone (1 mg) and estradiol (1 μg). The second and fourth pairs of thoracic glands were excised and cultivated for nine days as whole organs on perforated cyclopore membranes inserted into wells of culture plates in Waymouth's medium in an atmosphere of 50% O_2/5% CO_2. The mammary glands were then cultured in serum-free media supplemented with aldosterone, ovine prolactin, insulin and hydrocortisone (APIH medium) to induce lobuloalveolar development. Involution was then initiated by withdrawing the lactogenic hormones (i.e., aldosterone, hydrocortisone and prolactin)[18].

To begin to isolate the differentially expressed genes (i.e., between "control" lactating glands and "test" involuting tissues), poly(A)+ mRNA was isolated from control and test tissues using the FasTrack isolation kit. Test and control libraries have been constructed using the SuperScript plasmid system (Life Technologies) and the respective cDNAs ligated to pSPORT1 and pSPORT2 plasmid as described[19]. The plasmids were then electroporated (ElectroMax™ DH125 cells) and dilutions of the transformed cells plated.

To isolate the differentially expressed genes, ssDNA and biotinylated RNA have been generated as described by Li et al[19]. These samples are now being prepared for the first round of subtractive hybridization.

III. CONCLUSIONS

Work has proceeded as planned to begin isolating differentially expressed gene products. Coincident with these experiments, conditioned media is being prepared from the involuting tissues in an attempt to generate polyclonal antisera against secreted proteins.

IV. REFERENCES

1. Liotta, L.A., Steeg, P.S., and Stetler-Stevenson, W.G. Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. Cell 64:327-336, 1991.

2. MacDougall, J.R., and Matrisian, L.M. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. Cancer Metast Rev. 14:351-362, 1995.

3. Heppner, K.J., Matrisian, L.M., Jensen, R.A., and Rodgers, W.H. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J. Pathol. 149:273-282, 1996.

4. Wolf, c., Rouyer, N., Lutz, Y., Adida, C., Loriot, M., Bellocq, J.P., Chambon, P., and Basset, P. Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression. Proc. Natl. Acad. Sci. 90:1843-1847, 1993.

5. Okada, A., Bellocq, J.P., Rouyer, N., Chenard, M.P., Rio, M.C., Chambon, P., and Basset, P. Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc. Natl. Acad. Sci. 92:2730-2734, 1995.

6. Basset, P., Bellocq, J.P., Wolf, C., Stoll, I., Hutin, P., Limacher, J.M., Podhajcer, O.L., Chenard, M.P., Rio, M.C., and Chambon, P. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348:699-794, 1990.

7. Byrne, J.A., Tomasetto, C., Garnier, J.M., Rouyer, N., Mattei, M.G., Bellocq, J.P., Rio, M.C., and Basset, P. A screening method to identify genes commonly overexpressed in carcinomas and the identification of a novel complementary DNA sequence. Cancer Res. 55:2896-2903, 1995.

8. Tomasetto, C., Regnier, C., Moog-Lutz, C., Mattei, M.G., Chenard, M.P., Lidereau, R., Basset, P., and Rio, M.C. Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. Genomics 28:367-376, 1995.

9 Puente, X.S., Pendas, A.M., Llano, E., Velasco, G., and Lopez-Otin, C. Molecular cloning of a novel membrane-type matrix metalloproteinase from a human breast carcinoma. Cancer Res. 56:944-949, 1996.

10. Basset, P., Wolf, C., and Chambon, P. Expression of the stromelysin-3 gene in fibroblastic cells of invasive carcinomas of the breast and other human tissues: a review. Breast Cancer Res. Treatment 24:185-193, 1993.

11. Aou, A., Anisowicz, A., Hendrix, M.J.C., Thor, A., Neveu, M., Sheng, S., Rafidi, K., Seftor, E., and Sager, R. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. Science 263:526-529, 1994.

12. Sutherland, H., Dougherty, G., and Dedhar, S. Developmental biology and oncology: two sides to the same coin? New Biologist 2:970-973, 1990.

13. Cross, M., and Dexter, T.M. Growth factors in development, transformation, and tumorigenesis. Cell 64:271-280, 1991.

14. Li, F., Strange, R., Friis, R.R., Djonov, V., Altermatt, H.J., Saurer, S., Niemann, H., and Andres, A.C. Expression of stromelysin-1 and TIMP-1 in the involuting mammary gland and in early invasive tumors of the mouse. Int. J. Cancer 59:560-568, 1994.

15. Lund, L.R., Romer, J., Thomasset, N., Solberg, H., Pyke, C., Bissel, M.J., Dano, K., and Werb, Z. Two distinct phases of apoptosis in mammary gland involution: proteinase-independent and -dependent pathways. Development 122:181-193, 1996.

16. Talhouk, R.S., Bissell, M.J., and Werb, Z. Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. J. Cell Biol. 118:1271-1281, 1992.

17. Yang, Y. Spitzer, E., Kenney, N., Zschiesche, W., Li, M., Kromminga, A., Muller, T., Spener, F., Lezius, A., Verkamp, J.H., Smith, G.H., Salomon, D.S., and Grosse, R. Members of the fatty acid binding protein family are diffentiation factors for the mammary gland. J. Cell Biol. 127:1097-1109, 1994.

18. Atwood, C.S., Ikeda, M., and Vonderhaar, B.K. Involution of mouse mammary glands in whole organ culture: A model for studying programmed cell death. Biochem Biophys. Res. Comm. 207:860-867, 1995.

19. Li, W.B., Gruber, C.E., Lin, J.J., Lim, R., D'Alessio, J.M., Jessee, J.A. The isolation of differentially expressed genes in fibroblast growth factor stimulated BC_3H1 cells by subtractive hybridization. BioTechniques 16:722-729, 1994.