vated receptor or even in the targeted delivery of signaling molecules to the ligand-activated nuclear receptors. M-FABP, closely related with the FABP subfamily with "MDGI function," has not yet been shown to possess tumor suppressor and/or growth-inhibitory properties, but it is a good candidate. In contrast, the distantly related liver-type FABP, for example, stimulates the mitogenesis of hepatocytes (15).

In conclusion, the so-called *MDGI* gene and its gene product do not exist, but we observe MDGI function, because all FABPs with tumor suppressor and/or growth-inhibitory activity thus far are expressed in various cell types of the mammary gland, but not exclusively. To understand the underlying mechanisms is a challenge.

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References

1. Shi, Y. E., Ni, J., Xiao, G., Liu, Y. E., Fuchs, A., Yu, G., Su, J., Cosgrove, J. M., Xing, L., Zhang, M., Li, J., Aggarwal, B. B., Meager, A., and Gentz, R. Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, *MRG*. Cancer Res., *57:* 3084–3091, 1997.
2. Shimizu, F., Watanabe, T. K., Shinomiya, H., Nakamura, Y., and Fujiwara, T. Isolation and expression of a cDNA for human brain fatty acid-binding protein (B-FABP). Biochim. Biophys. Acta, *1354:* 24–28, 1997.
3. Theile, M., Seitz, S., Arnold, W., Jandrig, B., Frege, R., Schlag, P. M., Hänsch, W., Guski, H., Winzer, K. J., Barrett, J. C., and Scherneck, S. A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. Oncogene, *13:* 677–685, 1996.
4. Böhmer, F. D., Kraft, R., Otto, A., Wernstedt, C., Hellman, U., Kurtz, A., Müller, T., Rohde, K., Etzold, G., Lehmann, W., Langen, P., Heldin, C-H., and Grosse, R. Identification of a polypeptide growth inhibitor from bovine mammary gland. Sequence homology to fatty acid- and retinoid-binding proteins. J. Biol. Chem., *262:* 15137–15143, 1987.
5. Yang, Y., Spitzer, E., Kenney, N., Zschiesche, W., Li, M., Kromminga, A., Müller, T., Spener, F., Lezius, A., Veerkamp, J. H., Smith, G. H., Salomon, D. S., and Grosse, R. Members of the fatty acid binding protein family are differentiation factors for the mammary gland. J. Cell Biol., *127:* 1097–1109, 1994.
6. Specht, B., Bartetzko, N., Hohoff, C., Kuhl, H., Franke, R., Börchers, T., and Spener, F. Mammary derived growth inhibitor is not a distinct protein but a mix of heart-type and adipocyte-type fatty acid-binding protein. J. Biol. Chem., *271:* 19943–19949, 1996.
7. Huynh, H. T., Larsson, C., Narod, S., and Pollak, M. Tumor suppressor activity of the gene encoding mammary-derived growth inhibitor. Cancer Res., *55:* 2225–2231, 1995.
8. Celis, J. E., Ostergaard, M., Basse, B., Celis, A., Lauridsen, J. B., Ratz, G. P., Andersen, I., Hein, B., Wolf, H., Orntoft, T. F., and Rasmussen, H. H. Loss of adipocyte-type fatty acid-binding protein and other protein biomarkers is associated with progression of human bladder transitional cell carcinomas. Cancer Res., *56:* 4782–4790, 1996.
9. Nordlund, J. J., and Farooqui, J. Z. Melanogenic Inhibitor, and Methods of Producing and Using the Same. International Patent, WO 94/12534, PCT/US93/11139, 1994.
10. Hohoff, C., Bleck, B., Binas, B., Börchers, T., van Tilbeurgh, H., and Spener, F. Gene structure and tertiary structure of epidermal fatty acid binding protein. Chem. Phys. Lipids, *88:* 124–125, 1997.
11. Nielsen, S. U., Rump, R., Højrup, P., Roepstorff, P., and Spener, F. Differentiational regulation and phosphorylation of the fatty acid-binding protein from rat mammary epithelial cells. Biochim. Biophys. Acta, *1211:* 189–197, 1994.
12. Nielsen, S. U., and Spener, F. Fatty acid-binding protein from rat heart is phosphorylated on Tyr19 in response to insulin stimulation. J. Lipid Res., *34:* 1355–1366, 1993.
13. Hresko, R. C., Bernier, M., Hoffman, R. D., Flores-Riveros, J. R., Liao, K., Laird, D. M., and Lane, M. D. Identification of phosphorylated 422(aP2) protein as pp15, the 15-kilodalton target of the insulin receptor tyrosine kinase in 3T3–L1 adipocytes. Proc. Natl. Acad. Sci. USA, *85:* 8835–8839, 1988.
14. Börchers, T., Unterberg, C., Rüdel, H., Robenek, H., and Spener, F. Subcellular distribution of cardiac fatty acid-binding protein in bovine heart muscle and quantitation with an enzyme-linked immunosorbent assay. Biochim. Biophys. Acta, *1002:* 54–61, 1989.
15. Sorof, S. Modulation of mitogenesis by liver fatty acid binding protein. Cancer Metastasis Rev., *13:* 317–336, 1994.
16. Page, R. D. M. TREEVIEW: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci., *12:* 357–358, 1996.

Reply

We reported the discovery of a novel human tumor growth inhibitor by differential cDNA sequencing (1). The predicted amino acid sequence of this novel tumor-suppressing factor has a significant sequence homology to H-FABP[1] (also known as MDGI) and thus was named *MRG*. *MRG* was found to be expressed in normal and benign human breast tissues but not in breast carcinomas. *MRG* has tumor-suppressing activities; it inhibits breast cancer cell growth *in vitro* and tumor growth *in vivo*.

Hohoff and Spener (2) suggest that *MRG* should be renamed B-FABP because: (*a*) the sequence of *MRG* was found to be identical to the recently deposited sequences of B-FABP in GenBank; and (*b*) the reported MDGI sequence is a mixture of H- and A-FABP, and the MDGI function is exerted by H-FABP. *MRG* was identified as a putative tumor suppressor gene in the mammary gland by a differential cDNA sequence but not by a FABP sequence homology search. We realized in the original study that the so-called bovine *MDGI* gene does not exist but represents a mixture of H- and A-FABP. Cellular FABPs are a highly conserved family of proteins consisting of several subtypes and have been suggested to be involved in intracellular fatty acid metabolism and trafficking. Among them, only H-FABP/MDGI and the recently identified MRG/B-FABP have MDGI-like tumor-suppressing activity against breast cancer. In the phylogenetic tree of the FABP family, Hohoff and Spener (2) also included A-FABP and epidermal-type FABP as the genes with MDGI-like function. However, no tumor-suppressing activity toward breast cancer has been reported for A-FABP and epidermal-type FABP. Although it has been reported that the loss of A-FABP expression was correlated with bladder cancer progression (3), A-FABP was also reported to stimulate the proliferation of myoblasts (4).

As members of the FABP family, the most characterized biological functions for H-FABP and B-FABP are tumor-suppressing activities against breast cancer. These include: (*a*) the loss of H-FABP/MDGI (5) and B-FABP/MRG expression (1) is associated with breast cancer progression; (*b*) the loss of MDGI (5) and *MRG*[2] expression in breast carcinomas may be mediated through inactivation of the promoters by hypermethylation in breast cancer cells; (*c*) both MDGI (6–8) and *MRG*[2] are highly expressed in the fully differentiated lactating mammary gland and induce mammary gland differentiation; (*d*) MDGI and *MRG* have been mapped to the chromosomes 1p35 (9) and 6q22-23,[3] which harbor the putative tumor suppressor genes for breast cancer (10, 11); and (*e*) both MDGI and *MRG* strongly suppress the growth of breast tumors (1, 9). Based on these well-established mammary gland and mammary tumor functions, I suggest keeping the names MDGI and *MRG* when referring to their functions on the mammary gland, and using H-FABP and B-FABP when referring to their well-accepted FABP family phylogenetic tree.

H-FABP/MDGI and B-FABP/MRG reveal no sequence homology to any of the hitherto known growth inhibitors. Although the mechanism(s) underlying the tumor-suppressing activity for MDGI/H-FABP and MRG/B-FABP is currently unknown, MDGI and *MRG* may inhibit the growth of breast cancer cells by inducing the differentiation of mammary epithelial cells. We recently demonstrated that: (*a*) *MRG* overexpression induced differentiation leading to lipid production in MDA-MB-231 human breast cancer cells;[2] and (*b*) *MRG*

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[1] The abbreviations used are: H-FABP, heart-type fatty acid-binding protein; MDGI, mammary-derived growth inhibitor; *MRG*, MDGI-related gene; FABP, fatty acid-binding protein; B-FABP, brain-type FABP; A-FABP, adipocyte-type FABP.

[2] G. Xiao, M. Wang, Y. E. Liu, and Y. E. Shi. Induced differentiation of breast cancer cells by mammary-derived growth inhibitor-related gene (*MRG*), whose expression is lost epigenetically in breast cancer cells, submitted for publication.

[3] GenBank accession number U51338.

was immunohistochemically overexpressed in the normal lobule epithelial cells from lactating women as compared with nonlactating women.[2] In this regard, MRG/B-FABP is a candidate mediator of the differentiating effect of pregnancy and lactation on breast epithelial cells. Epidemiological studies indicate that breast cancer develops more frequently in those who are nulliparous or late parous (12–15); lifetime lactation also favors a low risk of breast cancer (14–15). These results suggest that pregnancy and lactation-induced differentiation is protective against breast cancer. Manipulation of these processes by technologically simple and practical means is a major means for breast cancer prevention. The possibility of preventing breast cancer by treating young nulliparous females with hormones that induce MRG/B-FABP expression and mimic a full-term pregnancy warrants further investigation.

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References

1. Shi, Y. E., Ni, J., Xiao, G., Liu, Y. E., Fuchs, A., Yu, G., Su, J., Cosgrove, J. M., Xing, L., Zhang, M., Li, J., Aggarwal, B. B., Meager, A., and Gentz, R. Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibition-related gene, *MRG*. Cancer Res., *57:* 3084–3091, 1997.
2. Hohoff, C., and Spener, F. Correspondence re: Y. E., Shi *et al.*, Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, *MRG*. Cancer Res., *57:* 3084–3091, 1997. Cancer Res., *58:* 4015–4016, 1998.
3. Celis, J. E., Ostergaard, M., Basse, B., Celis, A., Lauridsen, J. B., Ratz, G. P., Andersen, I., Hein, B., Wolf, H., Orntoft, T. F., and Rasmussen, H. H. Loss of adipocyte-type fatty acid-binding protein and other protein biomarkers is associated with progression of human bladder transitional cell carcinomas. Cancer Res., *56:* 4782–4790, 1996.
4. Prinsen, C. F., and Veerkamp, J. H. Transfection of L6 myoblasts with adipocyte fatty acid-binding protein cDNA does not affect fatty acid uptake but disturbs lipid metabolism and fusion. Biochem. J., *329:* 265–273, 1998.
5. Huynh, H., Alpert, L., and Pollak, M. Silencing of the mammary-derived growth inhibitor (MDGI) gene in breast neoplasms is associated with epigenetic changes. Cancer Res., *56:* 4865–4870, 1996.
6. Kurta, A., Vogel, F., Funa, K., Heldin, C. H., and Grosse, R. Developmental regulation of mammary-derived growth inhibitor expression in bovine mammary tissue. J. Cell Biol., *110:* 1779–1789, 1990.
7. Binas, B., Spitzer, E., Zschiesche, W., Erdmann, B., Kurtz, A., Muller, T., Niemann, C., Blenau, W., and Grosser, R. Hormonal induction of functional differentiation and mammary-derived growth inhibitor expression in cultured mouse mammary gland explants. In Vitro Cell Dev. Biol., *28A:* 625–634, 1992.
8. Yang, Y., Spitzer, E., Kenney, N., Zschiesche, W., Li, M., Kromminga, A., Muller, T., Spener, F., Lezius, A., and Veerkamp, J. H. Members of the fatty acid binding protein family are differentiation factors for the mammary gland. J. Cell Biol., *127:* 1097–1108, 1994.
9. Huynh, H. T., Larsson, C., Narod, S., and Pollak, M. Tumor suppressor activity of the gene encoding mammary-derived growth inhibitor. Cancer Res., *55:* 2225–2231, 1995.
10. Bieche, I., Champeme, M. H., and Lidereau, R. A tumor suppressor gene on chromosome 1p32–pter controls the amplification of *MYC* family genes in breast cancer. Cancer Res., *54:* 4274–4276, 1994.
11. Theile, M., Seitz, S., Arnold, W., Jandrig, B., Frege, R., Schlag, P. M., Hansch, W., Guski, H., Winzer, K. J., Barrett, J. C., and Scherneck, S. A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. Oncogene, *13:* 677–685, 1996.
12. Love, R. R., and Vogel, V. G. Breast cancer prevention strategies. Oncology (Basel), *11:* 161–168, 1997.
13. Canty, L. Breast cancer risk: protective effect of an early first full-term pregnancy *versus* increased risk of induced abortion. Oncol. Nurs. Forum., *24:* 1025–1031, 1997.
14. Freudenheim, J. L., Marshall, J. R., Vena, J. E., Moysich, K. B., Muti, P., Laughlin, R., Nemoto, T., and Graham, S. Lactation history and breast cancer risk. Am. J. Epidemiol., *146:* 932–938, 1997.
15. Enger, S. M., Ross, R. K., Paganini-Hill, A., and Bernstein, L. Breastfeeding experience and breast cancer risk among postmenopausal women. Cancer Epidemiol. Biomark. Prev., *7:* 365–369, 1998.