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Letter to the Editor

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

Letter

We would like to draw attention to the identity of the transcript of the MDGI[1]-related gene, *MRG* (1). The corresponding cDNA was found by us to be 100% identical to that of the human B-FABP. Recently, three groups, including our own, deposited the human B-FABP cDNA sequence to GenBank.[2–4] Considering the well-accepted FABP nomenclature, *i.e.*, referring to the tissues of first detection and adding "-type" to stress that a FABP may be expressed in another tissue as well, B-FABP should be chosen for proper classification. For the corresponding gene notations, see Fig. 1. The initiation codon is $A_{16}TG$ [enumeration according to Shi *et al.* (1)], and the protein is comprised of 132 amino acids.

Shi *et al.* (1) detected the *MRG* transcript, now identified as B-FABP mRNA, in Northern blots of mRNA from the brain, skeletal muscle, and heart by using the full-length cDNA as a probe; Shimizu *et al.* (2) found by application of a probe derived from the 3′ untranslated region of the human B-FABP cDNA a transcript expressed in brain and skeletal muscle only (2), although both groups used commercially available (from Clontech) Multiple Tissue Northern Blots with polyadenylated RNA from human adult tissues preblotted onto the membrane. Moreover, two expressed sequence tags represent B-FABP in fetal heart[5] and B-FABP in pregnant uterus,[6] and a full-length cDNA cloned from retina[3] is identical to B-FABP cDNA as well. Obviously, the scope of B-FABP expression in man is not fully exploited as yet.

We cloned and heterologously expressed human B-FABP, and we identified polyunsaturated fatty acids of the ω-3 type as ligands having the highest affinities[7] for this protein. It remains to be elucidated whether the protein itself inhibits tumor growth or mediates the antitumor effect of ω-3 fatty acids. In this context, it is interesting to note that the human B-FABP gene has been mapped to chromosome 6q22–23,[3] a locus that has been reported to harbor a putative tumor suppressor gene for breast cancer (3).

We would also like to draw attention to the important point that within the phylogenetic tree of intracellular lipid-binding proteins (Fig. 1), B-FABP belongs to a closely related subfamily of proteins that act as tumor suppressors and/or growth inhibitors. This extraordinary feature for such a protein was first described by Grosse *et al.* (4, 5), who reported that a so-called "mammary-derived growth inhibitor" (MDGI), when added exogenously, inhibits the growth of breast carcinoma cells (4) and then that of mammary epithelial cells (5). Later, it turned out that MDGI is a mixture of H-FABP and A-FABP, both of which are expressed in the mammary gland (6). H-FABP fully replaced the MDGI effect and

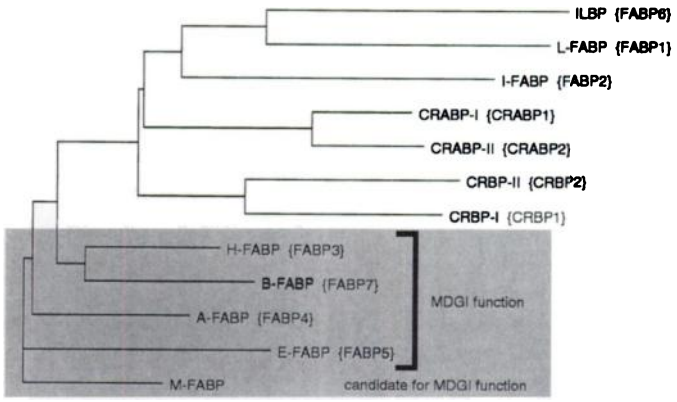

Fig. 1. Phylogenetic tree of the family of known human intracellular lipid-binding proteins. The branch length represents the evolutionary distance between the proteins. Human sequences were used for the calculation of the phylogenetic order using CLUSTAL [implemented in the program package HUSAR 4.0 (Deutsches Krebsforschungzentrum, Heidelberg, Germany)] and TreeView (16) for display. The gene notation is given in parentheses as assigned by the human genome database. *ILBP*, ileal lipid-binding protein; *L-FABP*, liver-type FABP; I-FABP, intestinal-type FABP; *CRABP*, cellular retinoic acid-binding protein; *CRBP*, cellular retinol-binding protein. The FABP subfamily with MDGI function is *shaded*, and B-FABP (identical to the *MRG* gene) is marked in *bold*.

inhibited the growth of normal epithelial cells but not that of normal stromal cells, an effect that could be fully mimicked by the 11-amino acid COOH-terminal peptide of H-FABP (5). Nevertheless, Huynh *et al.* (7) demonstrated that the human MCF-7 breast cancer cell line transfected with bovine H-FABP showed reduced growth compared with that of normal and mock-transfected MCF-7 cells. By the same token, loss of A-FABP was correlated with the progression of human bladder transitional cell carcinoma (8), and E-FABP was reported to selectively inhibit tyrosinase activity and cellular proliferation of melanoma cells, whereas normal skin fibroblasts were unaffected (9).

All proteins shown in the phylogenetic tree of the gene family of intracellular lipid-binding proteins (Fig. 1) share a common tertiary fold, *i.e.*, the β-barrel motif composed of two orthogonal β-sheets with five antiparallel β-strands each, to form the binding cavity that is capped by a helix-turn-helix motif. Within this gene family, H-, A-, E-, B- and M-FABP form a subfamily characterized by additional common features: (*a*) the crystal structures of the holo proteins, including those of the more recently discovered E-FABP (10) and B-FABP,[8] reveal a typical U-shaped conformation of one molecule of long-chain fatty acid bound in the cavity; and (*b*) the primary sequences contain a tyrosine residue within a consensus sequence for phosphorylation by a tyrosine kinase. Indeed, phosphorylation of Tyr-19 was shown for H-FABP in the mammary gland (11) and myocytes (12) as well as for A-FABP in adipocytes (13) on stimulation with insulin. Although intracellularly, these FABPs are mainly found in the cytosol, H-FABP has already been detected in the nucleus, albeit at lower concentrations (14). One may speculate that the nuclear FABP is involved in the control of concentrations of free fatty acids available as ligands and transcriptional activators to the peroxisome proliferator-acti-

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

[2] GenBank accession number D88648.

[3] GenBank accession number U51338.

[4] GenBank accession number AJ002962.

[5] GenBank accession numbers W72051 and W76403.

[6] GenBank accession number AA029795.

[7] F. Schnütgen, T. Börchers, N. Xhong, R. Godbout, J. C. Sacchettini, and F. Spener. Human B-FABP shows high affinity for ω-3 fatty acids but not for ω-6 fatty acids, manuscript in preparation.

[8] G. Scapin, F. Schnütgen, F. Spener, and J. C. Sacchettini. The three-dimensional structure of human B-FABP at 2.8 Å resolution, manuscript in preparation.

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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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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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[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.

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