CORRESPONDENCE

Re: Effect of γ-Linolenic Acid on the Transcriptional Activity of the Her-2/neu (erbB-2) Oncogene

Menendez et al. (1) recently reported that γ-linolenic acid (GLA) suppresses the expression of the Her-2/neu (erbB-2) oncogene in several cancer cell lines in vitro and that concurrent treatments of Her-2/neu-overexpressing cancer cells with GLA and the anti-Her-2/neu antibody showed synergistic increases in apoptosis and reduced growth and colony formation. These findings are interesting but not surprising because it has previously been shown that several polyunsaturated fatty acids (PUFAs)—GLA, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)—can kill a variety of tumor cells without harming normal cells, although the sensitivity of tumor cells to the cytotoxic action of these fatty acids varied (2–4).

In those studies, GLA was the most potent compound; the more highly unsaturated compounds AA, EPA, and DHA were also effective but were much less selective. This finding is surprising because it was thought that GLA and other fatty acids induce apoptosis of tumor cells by a free radical–dependent mechanism and so the higher the unsaturation index, the higher the tumoricidal ability [reviewed in (4)]. In addition, GLA produced alterations in tumor cell membrane lipid composition and mitochondrial ultrastructure; induced a substantial decrease in the activity of mitochondrial respiratory chain complexes I + III, IV and mitochondrial membrane potential; increased cytochrome c release from mitochondria; and activated caspases and DNA fragmentation, leading to apoptosis of tumor cells [reviewed in (4)]. GLA also decreased the antioxidant content of tumor cells, suppressed the expression of the oncogene Ras and the antiapoptotic gene Bcl-2, and enhanced the expression of p53 (5). This action of GLA on gene expression could be related to the ability of its peroxidized products to bind to DNA (5).

The ability of GLA to suppress the expression of the Her-2/neu (erbB-2) oncogene could be due to the binding of GLA and/or its peroxidized products to DNA. Her-2/neu (erbB-2) reduces the apoptotic effects of N-(4-hydroxyphenyl)retinamide in breast cancer cells by decreasing production of the free radical nitric oxide (NO) (6). Further, the cyclooxygenase-2 (COX-2) product prostaglandin E2 reverses the induction of apoptosis and NO production induced by the combination of N-(4-hydroxyphenyl)retinamide and COX-2 inhibitor in breast cancer cells (7). This finding suggests that the Her-2/neu (erbB-2) oncogene behaves as an antioxidant and that the increased COX-2 activity seen in breast cancer cells is a protective mechanism developed by tumor cells to escape from the tumoricidal action of PUFAs. This observation implies that the activity of fatty acid-CoA ligase (FACL), another PUFA-utilizing enzyme, may be increased in drug-resistant tumor cells. The relative resistance of cancer cells to apoptosis could be due to overexpression of COX-2 and FACL, which could serve as “sinks” for unesterified PUFAs. If so, reduction of apoptosis could be inversely correlated with the cellular level of PUFAs. The apoptotic response of tumor cells that overexpress FACL and COX-2 could be restored by the addition of nonsteroidal anti-inflammatory drugs and PUFAs. Thus, it is likely that one mechanism by which trastuzumab inhibits tumor growth is by enhancing free radical generation by blocking the Her-2/neu (erbB-2) oncogene. This mechanism could explain why a combination of GLA and trastuzumab led to synergistic increases in apoptosis.

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REFERENCES


RESPONSE

We welcome the correspondence by Dr. Das on our recent report that the ω-6 fatty acid (FA) γ-linolenic acid (GLA; 18:3n-6) transcriptionally suppresses the Her-2/neu oncogene (1). Although his molecular explanation for the synergism between GLA and trastuzumab is conceptually correct, it is unlikely that binding of GLA and/or its peroxidized products to DNA could explain this interaction.

Specifically, we do not agree with Das’s suggestion that a general mechanism involving the generation of lipoperoxides probably explains Her-2/neu suppression by GLA. Oleic acid (OA; 18:1n-9), the main ω-9 FA in olive oil, is monounsaturated whereas GLA is polyunsaturated. Nevertheless, OA is molecularly equivalent to GLA in that it suppresses Her-2/neu and interacts synergistically with trastuzumab (2,3). Treatment with GLA or OA increases the expression of the transcriptional repressor PEA3 in Her-2/neu gene–amplified tumor cells, and an intact PEA3 DNA-binding site on the endogenous Her-2/neu promoter is essential for GLA- and OA-induced Her-2/neu repression. Thus, a PEA3 binding site–mutated sequence impairs GLA- or OA-induced transcriptional blockade of Her-2/neu, whereas this effect does not occur when a Simian virus 40 viral promoter controls Her-2/neu expression (1,3). FAs other...
the TATA (minor (+1 base pair [bp]) and minor (−69 bp) transcription start sites of the Her-2/neu promoter are indicated, as are the positions of the TATA (−22 to −26 bp) and CCAAT (−71 to −75 bp) boxes and the relative positions of the major Ets transcription factor binding sites AP-2, PEA3, and ZONAB. If the Ets binding site is occupied by PEA3, then the TATA-binding protein will not be able to access the closely associated TATA box, thus repressing the Her-2/neu promoter. Cancer cells bearing the Her-2/neu gene amplification naturally express low to undetectable levels of PEA3 and, therefore, exhibit a PEA3 binding site–enhanced transcriptional activity of Her-2/neu promoter. Formation of inhibitory “PEA3–PEA3 DNA binding site” complexes at the Her-2/neu promoter ultimately links FAS inhibition–related accumulation of malonyl-CoA to transcriptional repression of Her-2/neu expression in GLA-treated Her-2/neu gene–amplified human cancer cells. ACC, acetyl-CoA carboxylase.

Fig. 1. Working model for GLA-induced transcriptional repression of Her-2/neu oncogene in cancer cells. Fatty acid synthase (FAS) produces the 16-carbon fatty acid (FA) palmitate through successive NADPH-dependent condensations of acetyl-coenzyme A (CoA) with malonyl-CoA. As a result of FAS blockade induced by exogenous FAs such as γ-linolenic acid (GLA), chemical FAS blockers, or small inhibitory RNA targeting the FAS gene, high levels of malonyl-CoA continue to be generated by acetyl-CoA carboxylase (ACC). Concomitant with FAS inhibition, the expression of PEA3, an Ets transcription factor that strongly attenuates the activity of Her-2/neu promoter, increases. The major (+1 base pair [bp]) and minor (−69 bp) transcription start sites of the Her-2/neu promoter are indicated, as are the positions of the TATA (−22 to −26 bp) and CCAAT (−71 to −75 bp) boxes and the relative positions of the major transcription factor binding sites AP-2, PEA3, and ZONAB. If the Ets binding site is occupied by PEA3, then the TATA-binding protein will not be able to access the closely associated TATA box, thus repressing the Her-2/neu promoter. Cancer cells bearing the Her-2/neu gene amplification naturally express low to undetectable levels of PEA3 and, therefore, exhibit a PEA3 binding site–enhanced transcriptional activity of Her-2/neu promoter. Formation of inhibitory “PEA3–PEA3 DNA binding site” complexes at the Her-2/neu promoter ultimately links FAS inhibition–related accumulation of malonyl-CoA to transcriptional repression of Her-2/neu expression in GLA-treated Her-2/neu gene–amplified human cancer cells. ACC, acetyl-CoA carboxylase.

that GLA and OA can also modulate Her-2/neu regardless of their saturation index, even in the presence of the antioxidant vitamin E (4).

Because the effect is independent of the saturation index, lipoperoxide generation is unlikely to underlie it. Indeed, inhibition of fatty acid synthase (FAS)–catalyzed de novo biogenesis of saturated FA similarly suppresses Her-2/neu overexpression and interacts synergistically with trastuzumab by inducing PEA3-dependent Her-2/neu repression (5). We have recently found that specific depletion of FAS protein after transient transfection with a small inhibitory RNA that targets the FAS gene strongly antagonizes the anti-Her-2/neu effects of GLA (Menendez JA, Lupu R: unpublished data). That is, GLA-induced inhibition of Her-2/neu promoter activity cannot occur in the absence of FAS. Considering the fact that GLA inhibits FAS efficiently (6), it is reasonable to suggest that FAS blockade is necessary and sufficient to promote Her-2/neu repression. In agreement with this assumption, bezafibrate, which blocks the activity of the rate-limiting enzyme of endogenous FA synthesis, acetyl-coenzyme A (CoA) carboxylase, did not reduce Her-2/neu promoter activity (Menendez JA, and Lupu RL: unpublished data). Conversely, the FAS substrate malonyl-CoA on its own statistically significantly decreased Her-2/neu promoter activity (Menendez JA, and Lupu R: unpublished data). Therefore, it appears that supraphysiological accumulation of malonyl-CoA, due to its reduced utilization by FAS in the presence of GLA, acts as an indicator of “cell fuel” availability that suppresses Her-2/neu via formation of inhibitory PEA3 protein–PEA3 DNA binding site complexes on the endogenous Her-2/neu promoter (Fig.1).

Finally, Das suggests that enhanced generation of free radicals could explain the synergism of GLA and trastuzumab because Her-2/neu directly participates in antioxidative processes. Our studies coincide with this suggestion but go further with the finding of Zhang et al. (7) that trastuzumab-sensitive proteins involved in metabolic and detoxification pathways, including FAS, are highly expressed in Her-2/neu–positive breast cancer. In view of all these findings, we consider Her-2/neu to be an “energy sensor” that actively participates in the response of cancer cells to nongenotoxic metabolic stresses, thus opening a new molecular avenue in the management of Her-2/neu–overexpressing carcinomas.

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NOTES

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DOI: 10.1093/jnci/djj203
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