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Effect of PPARα Ligand on TNFα-Dependent Expression of EGF Receptor in Human Glioma Cell Line

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Abstract: In glioma cells, tumor necrosis factor alpha (TNFα) was reported to increase the number of epidermal growth factor receptor (EGFR) that mediates EGF-dependent cell proliferation. On the other hand, ligands for peroxisome proliferator-activated receptor alpha (PPARα) have been reported to inhibit proliferation of various tumor cells. In this study, we evaluated the influence of synthetic PPARα ligand, Wy14643, on basal and TNFα-dependent expression of EGFR in U251MG cells derived from a human malignant glioma. Northern blot analysis revealed that TNFα increased EGFR mRNA levels and that this increase was markedly suppressed by Wy14643. However, it had only marginal effect on the basal levels of EGFR mRNA. These results demonstrate that the activation of PPARα leads to the inhibition of the TNFα-dependent EGFR expression in human malignant glioma cells.

Key words: glioma, EGFR, PPARα, TNFα

Overexpression of epidermal growth factor receptor (EGFR) mainly due to its gene amplification occurs frequently in diverse human carcinomas of the breast, ovary, cervix, kidney and squamous cells.[1-3] Such alteration is also observed in 40–50% of the human malignant gliomas.[4,5] The EGFR overexpression possibly leads to the promotion of tumor development by increasing the growth-stimulatory signaling by EGF. Several clinical and histopathological studies have shown that amplification of EGFR gene in malignant glioma is related to a shorter interval to relapse and poorer survival of the affected subjects.[6] On the other hand, it was also shown that tumor necrosis factor alpha (TNFα) increases the number of cell surface EGFR in some glioma cell lines.[7] Recently, efforts have been made to reduce the EGFR expression, thereby preventing the growth of tumor cells.[8-10]

PPARα (peroxisome proliferator-activated receptor alpha) is one of three subtypes of PPARs (α, β and γ) that are members of the ligand-dependent nuclear hormone receptor family, and plays an important role in lipid homeostasis.[11] Some synthetic ligands are widely used as therapeutic drugs for hyperlipidemia.[11] On the other hand, the anti-tumor effect of PPARα ligands has been reported in rat mammary tumor, early stages of colon tumorigenesis, and several human tumor cell lines (prostate carcinoma, melanoma).[12-14]

Hence, we demonstrated the expression of PPARα in human malignant glioma cell lines, U251MG and SK-MG-1.[15] Here we reported the influence of synthetic PPARα ligand, Wy14643, on basal and TNFα-dependent expression of EGFR mRNA in U251MG cells.

Materials and Methods

1. Cell culture

U251MG cells (human cell line derived from malignant glioma) were obtained from Memorial Sloan Kettering Cancer Institute (New York, USA). The cells were seeded in 100-mm dishes and cultured to confluence in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin) and 2mM L-glutamine at 37 °C in a humidified atmosphere (95% air and 5% CO_2). The cells were then treated with PPARα ligand, Wy14643, at a final concentration of 10 μM together with or without TNFα (final concentration of 100 U/ml). After 18-hour incubation, the cells were harvested for RNA extraction.

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2. Northern blot analysis

Total RNA was extracted from U251MG cells by the acid guanidine phenol/chloroform method.[16] After electrophoresis of 10 μg of total RNA on 0.8% agarose gel, the RNA was blotted on a nylone membrane (GeneScreen Plus, NEN, Boston, USA). The human EGFR cDNA probes were cloned by reverse transcription-coupled polymerase chain reaction (RT-PCR) using total RNA from U251MG cells. The sequences of oligonucleotides used were as follows : EGFR ; sense (the nucleotide positions 1116-1141 in accession number XM_004738) 5′-CGACAGCTATGAGATGGAGGAAGACG-3′, antisense (2139-2164) 5'-CCAGGGCCACCACCAGCAGC AAGAGG-3′. An amplified cDNA fragment (1049bp) was subcloned into pGEM-T Easy vector (Promega, Madison, USA). Authenticity of the cDNA was verified by sequencing. The probes were labeled with ^{32}P-dCTP using a random prime labeling kit (Roche Diagnostics, Tokyo, Japan). After the hybridization with the EGFR probes, radioactivities of the bands for the mRNA were quantified using BAS 2000 bioimage analyzing system (Fuji Film, Tokyo, Japan). The detailed conditions for Northern blot analysis were described previously.[17]

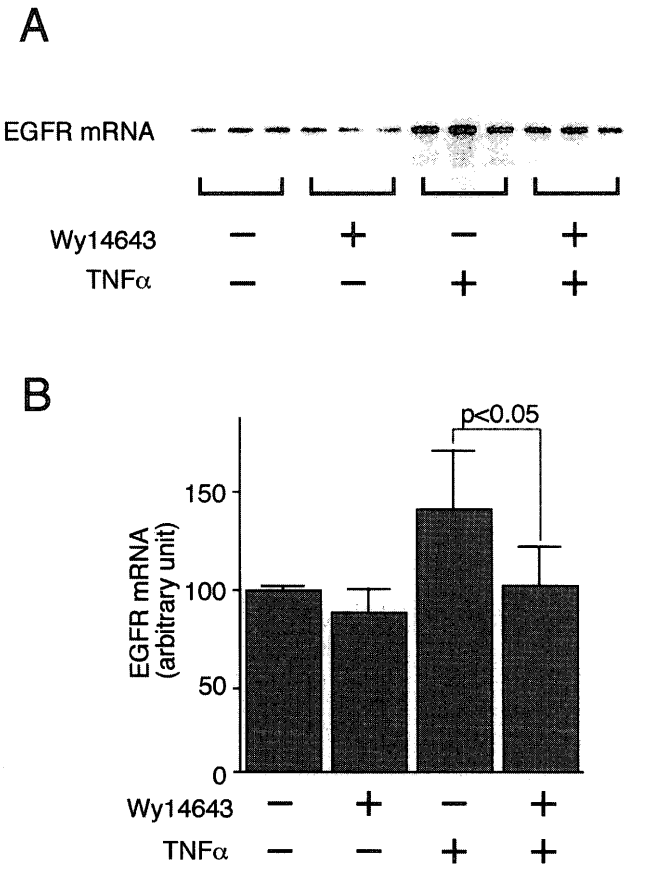

Fig. 1 Wy14643 inhibits TNFα-dependent induction of EGFR mRNA. U251MG cells were treated with Wy14643 (10 μM) alone or together with TNFα (100 U/ml). After 18-hour incubation, the cells were harvested, and Northern blot analysis was performed using EGFR cDNA as a probe. Experiment was carried out in triplicate (**A**). The radioactivities of bands were quantified using BAS2000 bioimage analyzing system and shown as arbitrary units: the means ± standard deviation (**B**).

3. Statistical analysis

Statistical analysis was carried out using one-way ANOVA followed by Fisher's protected least significant difference (PLSD) analysis and a p value less than 0.05 was considered significant.

Results

A single band at 8.5 kb for EGFR mRNA was detected by Northern blot analysis as shown in Figure 1. The size was compatible with the previous report by Harper et al.[18] Expression of EGFR mRNA was observed in the basal condition, indicating its constitutive expression in U251MG cells. The levels were not altered by the treatment of the cells with PPARα ligand, Wy14643 for 18 hours. Treatment with TNFα significantly increased the levels of EGFR mRNA. This increase was suppressed significantly by Wy14643.

Discussion

The present study demonstrated that the activation of PPARα by its synthetic ligand, Wy14643, leads to the inhibition of the TNFα-dependent increase in EGFR expression in human malignant glioma U251MG cells. However, Wy14643 alone has no effect on the basal expression of EGFR mRNA. These findings may indicate that the inhibitory effect of Wy14643 is specific to TNFα-activated intracellular signaling pathway. TNFα exerts its effects through the activation of transcription factor NF-κB. In unstimulated cells, NF-κB is present in the cytoplasm, binding to its inhibitory protein IκB. TNFα stimuli result in the degradation of IκB, leading to the translocation of NF-κB into the nucleus, where it activates its target genes. Recently, Delerive et al. reported that synthetic PPARα ligand induces the expression of IκBα (one of major IκBs) in human aortic smooth muscle cells and in primary human hepatocytes, and inhibits the nuclear translocation of NF-κB.[19] Although it is still remained to be studied whether TNFα-dependent EGFR expression in glioma cells is mediated by the NF-κB activation or not, PPARα-dependent accumulation of IκBα is a possible mechanism for the inhibition of the TNFα-dependent EGFR expression in U251MG cells.

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