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Research Report

N-(4-Hydroxyphenyl) retinamide induced both differentiation and apoptosis in human glioblastoma T98G and U87MG cells

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ABSTRACT

N-(4-Hydroxyphenyl) retinamide (4-HPR) is a synthetic retinoid that has shown biological activity against several malignant tumors and minimal side effects in humans. To explore the mechanisms underlying the chemotherapeutic effects of 4-HPR in glioblastoma, we used two human glioblastoma T98G and U87MG cell lines. *In situ* methylene blue staining showed the morphological features of astrocytic differentiation in glioblastoma cells following exposure to 1 μM and 2 μM 4-HPR for a short duration (24 h). Astrocytic differentiation was associated with an increase in expression of glial fibrillary acidic protein (GFAP) and downregulation of telomerase. Wright staining and ApopTag assay indicated appearance of apoptotic features in glioblastoma cells following exposure to 1 μM and 2 μM 4-HPR for a long duration (72 h). We found that 4-HPR caused apoptosis with activation of caspase-8 and cleavage of Bid to truncated Bid (tBid). Besides, apoptosis was associated with alterations in expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins resulting in an increase in Bax:Bcl-2 ratio, mitochondrial release of cytochrome c and Smac, downregulation of selective baculoviral inhibitor-of-apoptosis repeat containing (BIRC) molecules, an increase in intracellular free [Ca^{2+}], and activation of calpain and caspase-3. Taken together, these results strongly suggested that 4-HPR could be used at low doses for induction of both differentiation and apoptosis in human glioblastoma cells.

1. Introduction

Chemotherapeutic approaches to glioblastoma, which is the most malignant brain tumor, are presently not successful because of significant toxicity, problems with drug delivery, and the high degree of drug-resistance. There are many clinical trials evaluating emerging therapeutic agents for the treatment of newly diagnosed glioblastoma patients. New agents that target cell characteristics such as differentiation, angiogenesis, invasion, DNA repair, and apoptosis and that show acceptable side-effect profiles are presently being investigated for their efficacy against this malignancy (Yung, 1994).

N-(4-Hydroxyphenyl) retinamide (4-HPR), also known as fenretinide, is a synthetic derivative of all-*trans* retinoic acid (ATRA) and it induces apoptosis in cancer cell lines, shows minimal side effects in humans and also does not accumulate in the liver (Costa et al., 1995). It is also one of the most

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promising retinoids in both cell culture and animal models of cancers. Over 25 years ago, 4-HPR proved to be a potent inhibitor of mammary carcinogenesis in the rats (Moon et al., 1979). Since then, 4-HPR has been studied extensively and found to be less toxic and less genotoxic than other retinoids. The Chemoprevention Branch of the National Cancer Institute has active 4-HPR trials for cancers in several organ sites including the prostate, lung, oral cavity, breast, bladder, and cervix (Zou et al., 2003). It has also been found to be highly growth inhibitory in cervical cancer, ovarian cancer, endometrial cancer, lung cancer, non-small cell lung cancer, head and neck squamous cell carcinoma, esophageal carcinoma, prostate cancer, breast carcinoma, colon carcinoma, kidney carcinoma, bladder carcinoma, neuroblastoma, leukemia, non-M3 acute myeloid leukemia, NB4 acute promyelocytic cells, transformed cells such as NIH 3T3 mouse fibroblasts, and F9 embryonal carcinoma cells (Zou et al., 2003).

Moreover, 4-HPR is a valuable tool for defining apoptotic signaling pathways and understanding the mechanisms of synergy with other chemotherapeutic drugs. It has been reported earlier that 4-HPR reduces the expression of human telomerase reverse transcriptase (hTERT) catalytic subunit (Soria et al., 2001), suggesting that hTERT may represent a specific molecular marker for the detection of pre-invasive disease in early carcinogenesis and a potential intermediate biomarker to evaluate the efficacy of chemopreventive agents.

The mechanisms by which 4-HPR exerts its apoptotic effects are not yet clear. Unlike ATRA, 4-HPR induces its apoptotic effects mainly via retinoid receptor-independent mechanisms (Lippman et al., 2000). 4-HPR inhibits cell growth by inducing apoptosis in numerous tumor cell types including ATRA-resistant tumor cells (Ulukaya et al., 2003). However, the signaling mechanisms by which 4-HPR mediates its anti-proliferative effects remain unclear. On the basis of previous reports that 4-HPR induces both differentiation and apoptosis, we have hypothesized that 4-HPR may demonstrate those activities against human glioblastoma cells. Therefore, we examined the therapeutic efficacy of 4-HPR against human glioblastoma T98G and U87MG cells.

We examined induction of both differentiation and apoptosis in glioblastoma T98G and U87MG cells following exposure to two low doses (1 μM and 2 μM) of 4-HPR for a short time-point (24 h) and a long time-point (72 h). Both doses of 4-HPR for a short exposure (24 h) induced only differentiation but for a long exposure (72 h) caused apoptosis in both glioblastoma cell lines. Induction of apoptosis involved initial caspase-8 activation followed by late mitochondrial release of cytochrome *c* and minor caspase-9 activation, suggesting that caspase-8 activation was the major trigger for apoptosis. Production of pro-apoptotic tBid and overexpression of pro-apoptotic Bax occurred in the course of apoptosis. In contrast, levels of anti-apoptotic BIRC-2 to BIRC-6 expression were decreased to favor apoptosis. Taken together, these results indicated that 4-HPR at low doses could be used as a potential chemotherapeutic drug for induction of both differentiation and apoptosis in glioblastoma cells depending on duration of treatment time.

2. Results

2.1. 4-HPR induced differentiation with overexpression of GFAP and downregulation of telomerase

Low doses (1 μM and 2 μM) of 4-HPR for a short time (24 h) suppressed cell proliferation and induced morphological and biochemical features of astrocytic differentiation in both T98G and U87MG cells (Fig. 1). *In situ* methylene blue staining showed growth restriction and morphology of astrocytic differentiation such as appearance of thin cells with long

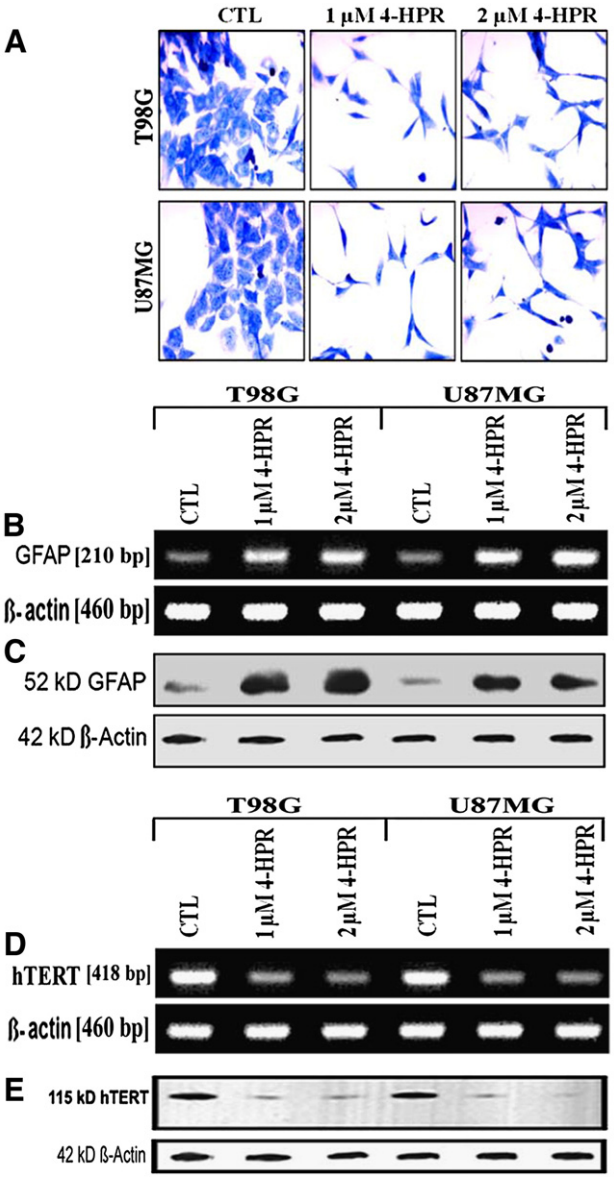

Fig. 1 – Detection of morphological and biochemical features of astrocytic differentiation in T98G and U87MG cells. Treatments (24 h): control (CTL), 1 μM 4-HPR, and 2 μM 4-HPR. (A) Methylene blue staining to detect astrocytic morphology. Determination of GFAP expression by (B) RT-PCR and (C) Western blotting. Determination of hTERT expression by (D) RT-PCR and (E) Western blotting.