

# IFN- $\gamma$ Regulates Expression of BRG1 Associated Factor 155/170 and Sensitivity to Steroid in Astrocytes

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## ABSTRACT

**Background:** The expression of BRG1 associated factors (BAF) 155 and BAF 170 in response to IFN- $\gamma$  or TNF- $\alpha$  was studied in astrocytoma cell lines and primary astrocytes. BAFs are complexed with BRG1 and are also associated with activated glucocorticoid for glucocorticoid trans-activation. **Methods:** IFN- $\gamma$  was pretreated for 18 hrs and cells were incubated with IL-1 or TNF- $\alpha$  for 72 hrs or 96 hrs with different concentrations of steroid. Cell death was measured by LDH assay. BAF expression was assayed by RT-PCR. **Results:** IFN- $\gamma$  increased cell death by dexamethasone in LN215 cells but not in LN319 cells. The IFN- $\gamma$  increased the expression of BAF 155 and BAF 170 in adult astrocytes and LN215 cells, but IFN- $\gamma$  decreased the expression of BAF 155/170 in LN319 cells. The effect of IFN- $\gamma$  on the expression of BAF was not as clear in fetal astrocytes as it was in adult astrocytes. **Conclusion:** Our results suggest cytokines produced during immune reaction or immunotherapy may modulate steroid susceptibility of astrocytes and astrocytoma cells by influencing the expression of BAFs. (*Immune Network* 2004;4(4):224-228)

**Key Words:** Astrocyte, steroid, BAF155/170, IFN- $\gamma$

## Introduction

Astrocytes represent the major portion of CNS (central nervous system) cells. Their main role is to physically protect neurons, but the function of astrocytes has been studied with respect to immunologic activity, such as MHC class II endocytic pathway (1). When inflammation, tumor progression or a degenerative disease occurs in the CNS, T lymphocytes infiltrate and secrete IFN- $\gamma$  or TNF- $\alpha$ , which primes further immune reactions of the astrocytes.

Astrocytoma is the most common brain tumor, and can be treated by steroid hormone therapy. In brain tumors, the metabolism of steroids is challenged (2). Moreover, steroid hormone receptors are found in astrocytomas, which are involved in the

regulation of specific genes (3-5). Such aberrant control of transcription may be an important aspect of astrocytes acquiring immortality in the transformed stage.

Several chromatin remodeling complexes have been identified in recent years. Most of these are related to the yeast SWI/SNF complex by virtue of the fact that they contain a subunit that is homologous to SWI2 (6). Both biochemical and genetic studies suggest that SWI/SNF complex antagonizes chromatin-mediated repression in yeast (7). The view that SWI/SNF-related complexes function similarly in other organisms is supported by the discovery of the BRM (Brahma) gene in *Drosophila*, which is 50% identical to SWI2/SNF2. Mammalian cells contain at least two genes, hBRM (human BRM) (8) and BRG (Brahma-related gene) (9), that are closely related to SWI2 and also to Brahma. Both BRG1 and hBRM are parts of independent complexes (9,10), which have nucleosome remodeling activity similar to that of the yeast complex (10)

BRG-1 complex is a human counterpart (11) of SNF/SWI complex of yeast. hbrm/in *drosophila* (12)

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has an ability to remodel chromatin with ATPase activity. In mammalian cells, BRG-1 is complexed with other proteins BAFs (BRG-1 associated factors) (10). BAF 155 and BAF 170 is a component of complex to transduce the signals in glucocorticoid trans-activation (13). Co-immunoprecipitation showed that glucocorticoids promote a hormone-dependent association of glucocorticoid with hBRG1 complex (14).

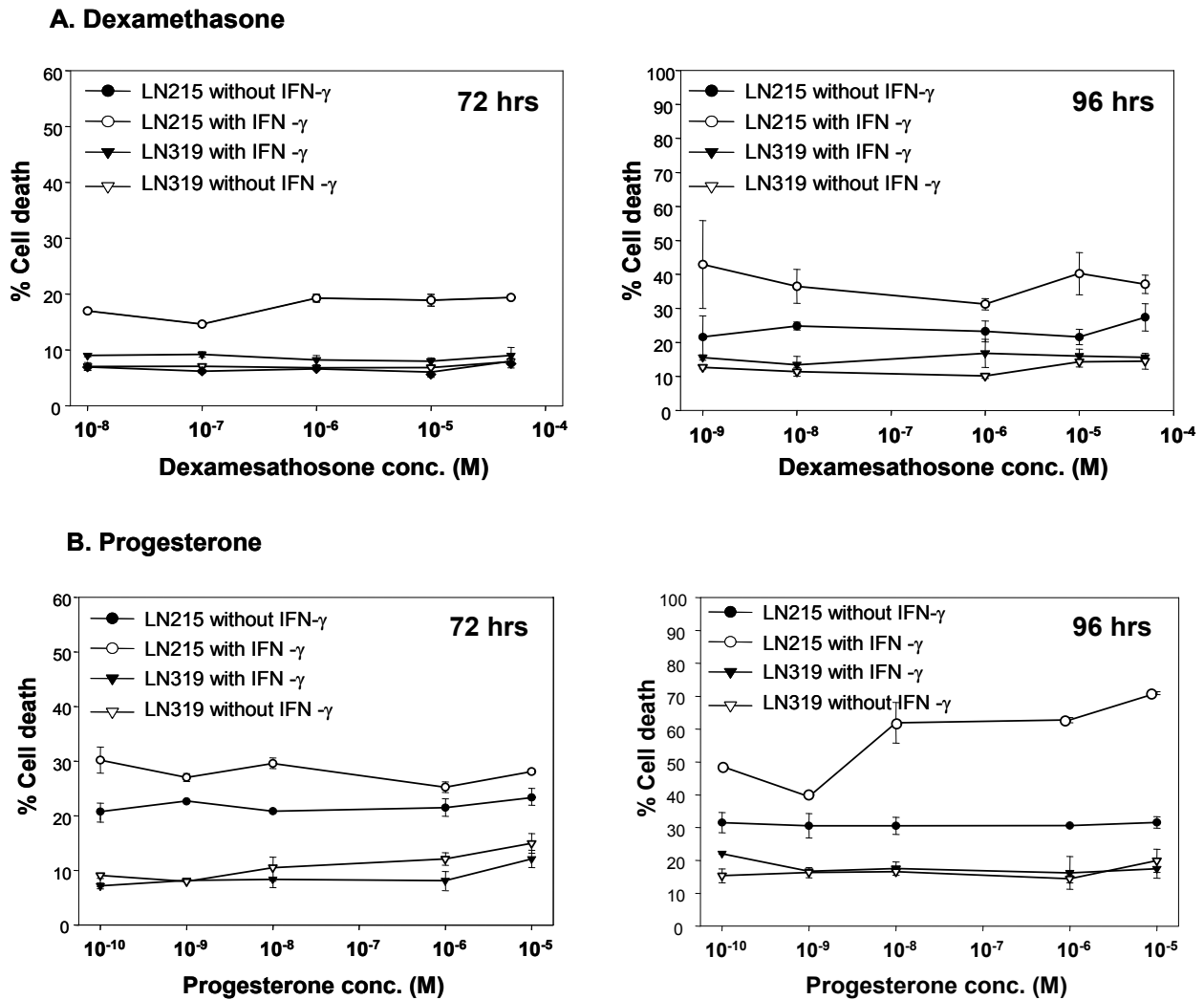
Steroid hormones act via a group of high affinity receptors that regulate transcription by binding to hormone response elements located within the promoters of hormone-inducible genes (15). The BRG-1 complex is a human counterpart (11) of the SNF/SWI complex of yeast. Similar component hbrm in drosophila (12) has an ability to remodel

Among several human astrocytoma cell lines, LN215 and LN319 were studied in this study. These two cell lines have unique characteristics in response to oxidative stress in our previous study. LN215 cells were more sensitive to oxidative stress than LN319 cells (15).

In this study the effect of pro-inflammatory cytokines on the expression of protein related to the chromatin remodeling complex in primary astrocytes or in astrocytoma cell lines was addressed. Therefore, the expressions of BAF 155 and BAF 170, the protein partners of the activated glucocorticoid were measured after treatment with TNF- $\alpha$  or IFN- $\gamma$ .

**Materials and Methods**

*Culture of astrocytoma cell lines and the preparation of*



**Figure 1.** Cell death induced by steroid in astrocytoma cell lines in response to IFN- $\gamma$ . IFN- $\gamma$  was pretreated for 18 hrs and cells were incubated with IL-1 or TNF- $\alpha$  for 72 hrs or 96 hrs with different concentrations of steroid. Cell death was measured by LDH assay.

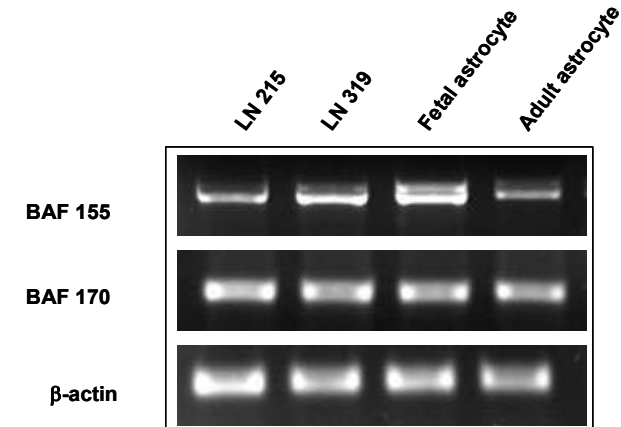
chromatin with ATPase activity (9).

*primary astrocytes.* Fetal astrocytes were isolated from

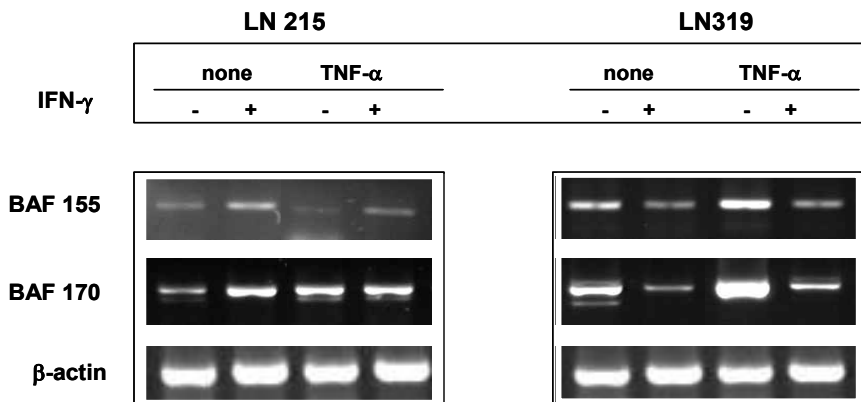
25-week-old human fetus and adult astrocytes were isolated from a 30-year-old female. The samples were obtained from therapeutic abortion or surgery for epilepsy, but they were from normal brain tissue in both cases. Astrocytes were cultured in 10% FCS (Gibco BRL, Grand Island, NY)-DMEM (Gibco BRL, Grand Island, NY) containing 1% nonessential amino acids (Sigma, St. Louis, MO). The cultures were maintained for up to two and half months. Indirect fluorescence staining for GFAP (glial fibrillary acidic protein) revealed that most of the cultured cells (>99%) were astrocytes. Astrocytoma cell lines, LN215 and LN319, were kindly provided by Dr. E. G. Van Meir (Department of Neurosurgery, Laboratory of Tumor Biology and Genetics, Lausanne, Switzerland) and maintained in 10% FCS containing 1% nonessential amino acids.

*Treatment of cytokines and steroids.* Cells were cultured with 750 U/ml of TNF- $\alpha$  (Genzyme, Cambridge, MA) for 24 hrs. IFN- $\gamma$  (Genzyme, Cambridge, MA) at 100 U/ml was pretreated for 18 hrs before adding the TNF- $\alpha$ . Dexamethasone (Sigma, St. Louis, MO) or progesterone (Sigma, St. Louis, MO),  $10^{-9}$  ~  $10^{-4}$  M, was added to the medium 18 hrs after IFN- $\gamma$

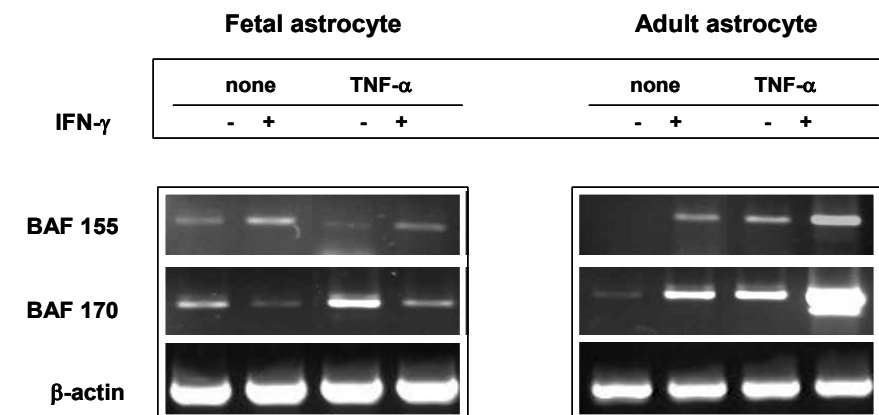
*Semi-quantitative reverse transcription PCR.* The level of BAF 155 and BAF 170 mRNA was measured by RT-PCR. Total RNA was isolated using an RNeasy kit (Quiagen, Valencia, CA). Total RNA, 1  $\mu$ g, was used to synthesize cDNA with 0.1 O.D. of random



**Figure 2.** Expression of BAF in astrocytoma cells and primary astrocytes. Semi-quantitative PCR was performed using  $\beta$ -actin as internal control.



**Figure 3.** Expression of BAF in astrocytoma cells in response to IFN- $\gamma$  or TNF- $\alpha$ . IFN- $\gamma$  was pretreated for 18 hrs and cells were incubated with TNF- $\alpha$  for 24 hrs. Semi-quantitative PCR was performed using  $\beta$ -actin as internal control.



**Figure 4.** Expression of BAF in primary astrocytes in response to IFN- $\gamma$ , or TNF- $\alpha$ . IFN- $\gamma$  was pretreated for 18 hrs and cells were incubated with TNF- $\alpha$  for 24 hrs. Semi-quantitative PCR was done using  $\beta$ -actin as an internal control.

treatment.

hexamer (Pharmacia, Uppsala, Sweden) and 200 U of

M-MLV reverse transcriptase (Gibco BRL, Grand Island, NY). RT-PCR was performed using the following primer sets; BAF 155 primers (forward: 5'-gCT ATC gTC TAA ACC CCC-3'; reverse: 5'-TgT TCA gTC CAT CCT CT-3'), BAF 170 primers (forward: 5'-Acg ggg AAC Aag ggA gAg-3'; reverse: 5'-gTA Tgg gTC TTC AAT ggg-3'). PCR conditions were as follows:- denaturation at 94°C for 30s, annealing at 57°C for 30s and extension at 72°C for 30s. PCR buffer was 10 mM Tris-HCl (pH 10), 2.0 mM MgCl<sub>2</sub>, 50 mM KCl with 1.25 U of Taq polymerase (Bioneer, Daejeon, Korea). After 20 cycles, an additional extension at 72°C for 10 min was carried out. BAF 155 primers recognized the region between 1,544 and 1,911 in BAF 155 (GenBank accession No.U66615) and BAF 170 primers recognized the region between 1,220 and 1,949 in BAF 170 (GenBank accession No.U66616). PCR products were cloned and their sequences confirmed by sequencing.  $\beta$ -actin was used as the internal control. *LDH assay*. 100 $\mu$ l of 0.3 mg/ml  $\beta$ -NADH and 25  $\mu$ l of 22.7 mM pyruvate were added to the culture supernatant or cells treated with Triton X-100. O.D. was read under A340 immediately.

## Results

*Effect of IFN- $\gamma$  in astrocytoma cells for susceptibility to dexamethasone or progesterone.* Dexamethasone did not induce cell death in LN215 or LN319 cells, but treatment with IFN- $\gamma$  induced cell death in LN215 cells at  $10^{-8}$ ~ $10^{-4}$  M of dexamethasone after 72 and 96 hrs respectively (Fig. 1A). Similar treatment with IFN- $\gamma$  induced cell death in LN215 cells at  $10^{-8}$ ~ $10^{-4}$  M of progesterone after 72 and 96 hrs respectively (Fig. 1B).

*Effect of IFN- $\gamma$  on the expression of BAF 155 and BAF 170 in primary astrocytes and astrocytoma cells.* Basal expression levels of BAF 155 and BAF 170 in astrocytoma cell lines were almost similar to fetal astrocytes, as determined by intensity measurements, after PCR amplification (Fig. 2). Primary cultures of adult astrocytes expressed less BAF 155 than fetal astrocytes (Fig. 2). In astrocytoma cell lines the expression of BAF was quite different in the two cell lines. In LN215 cells the expression of BAF 155 and BAF 170 increased with IFN- $\gamma$  treatment, as it did in primary cultures of adult astrocytes (Fig. 3). However, IFN- $\gamma$  treatment down regulated the BAF 155 and BAF 170 transcripts in LN319 cells. Expression patterns of BAFs in response to cytokines were different in the fetal and adult astrocytes. Although the basal expression level of BAF was low in adult astrocytes, the expressions of BAF 155 and BAF 170 were increased by IFN- $\gamma$  and TNF- $\alpha$ . In particular, the co-treatment with IFN- $\gamma$  and TNF- $\alpha$

increased BAF155/170 in adult astrocytes (Fig. 4). The overall response to cytokines in adult astrocytes was more prominent than in fetal astrocytes, in which BAF 170 mRNA transcript decreased after treatment with IFN- $\gamma$  or after co-treatment with IFN- $\gamma$  and TNF- $\alpha$  (Fig. 4).

## Discussion

Glucocorticoid regulates several genes in astrocytes, inhibits the proliferation of astrocytes, and complex effects of steroid agonists and antagonists on glioma cell proliferation have been reported (15,17,18). Glucocorticoid was found to down-regulate GFAP immunoreactivity, which may affect neurodegeneration (19,20). In rat astrocytoma cells glucocorticoid induced the expression of mRNA and the secretion of lipocortin (5). Steroid therapy has also been reported to achieve remarkable glioblastoma regression (21).

By adding IFN- $\gamma$ , cell death due to dexamethasone was increased in LN215 cells but not in LN319 cells. The intermediate molecules required during steroid signaling, BAF 155 or BAF 170, were induced in LN215 cells but not in LN319 cells. The BAF 155 and 170 molecules are similar, only the proline rich domain is different in the C-terminal of BAF 170 (14). In LN319 cells the transcripts of BAF 155 or BAF 170 were down-regulated by IFN- $\gamma$ . Although the mechanism of variable responses to steroid or the expression discrepancy of BAF to cytokines in each astrocytoma cell lines is not clear at present, the possibility that cytokines may alter cellular responses to steroid persists (22). SWI/SNF was reported to be required for IFN- $\gamma$  induction of CIITA, the master regulator of major histocompatibility complex class II expression (23). Despite the presence of functional STAT1, IRF-1 and USF-1, activators implicated in CIITA expression, IFN- $\gamma$  did not induce CIITA in cells lacking BRG1 and hBRM, the ATPase subunits of SWI/SNF. Reconstitution with BRG1 rescued CIITA induction (23). Although there are a few studies about IFN- $\gamma$  and hBRM, there is no report about TNF- $\alpha$  and hBRM until now.

The synthetic the glucocorticoid dexamethasone was reported as to elevate DR5 mRNA in glioblastoma, ovarian cancer, and colon cancer cell lines with mutant p53 undergoing apoptosis, and this induction is inhibited by the transcriptional inhibitor actinomycin D. Although another glucocorticoid, prednisolone, also induced apoptosis, it did not increase DR5 mRNA (24). Therefore, dexamethasone and prednisolone are likely to be different in terms of their apoptotic mechanisms. But in the present study, sensitivities to dexamethasone and prednisolone were similar in LN215 cells. After treatment

with IFN- $\gamma$  LN215 cells became more sensitive to both dexamethasone and prednisolone. Steroids induced more cell death in LN215 cells than in LN319 cells. This results is compatible to our previous data in which up-regulation of Fas/FasL by oxidative stress was shown in LN215 cells, not in LN319 cells (15).

As conclusions we suggest that 1) IFN- $\gamma$  increases the expression of BAF 155 and BAF 170 in adult astrocytes and LN215 cells, which then became sensitive to steroid after treatment of IFN- $\gamma$ , and 2) IFN- $\gamma$  decreased the expression of BAF 155 and BAF 170 in LN319 cells, which showed no notable changes upon steroid treatment with IFN- $\gamma$ . So the up-regulation of BAF might be the result of the malignant transformation from normal adult astrocytes to astrocytoma. Pro-inflammatory cytokines produced during the immune reaction may influence the steroid susceptibility of normal astrocytes and astrocytoma by modulating the expression of BAF 155 and BAF 170 mRNA.

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