

## A dance between interferon- $\alpha/\beta$ and p53 demonstrates collaborations in tumor suppression and antiviral activities

In the recent paper by Takaoka et al. (2003), the authors demonstrate that interferons- $\alpha$  and - $\beta$  stimulate p53 expression but not p53 activation. The increase in p53 expression translates into significant enhancement of apoptosis and reduction of chemotherapeutic dosages in vitro to destroy tumor cells. Furthermore, viral infections are also modulated by p53 in collaboration with interferons- $\alpha$  and - $\beta$ . These observations are significant and may lead to new paradigms for therapy if the high doses of interferon necessary to obtain the effects in vitro can be combined with more active interferons, interferons with minimal side effects, and/or novel delivery systems to target interferons directly to tumors.

The discovery, isolation, and successful clinical application of interferons marked a new era of biotechnology and medicine (Pestka et al., 1987). Discovery of the interferons originated from study of viral interference: how infection with one virus inhibited infection by another. The protein p53 was discovered during research on the virus SV40 (Thomas et al., 1983). Both the interferons and p53 have now earned a place in understanding the mechanisms of oncogenesis and in developing therapy for cancers. p53 is a tumor suppressor. Once activated, the p53 protein acts as a transcriptional regulator by binding to the promoter DNAs of various target genes that mediate its actions. For example, p53 induces expression of protein p21, which in turn directly blocks cell division by interaction with cdk2, a protein stimulating cell division. Mutations of p53 are found in most tumors; these mutations do not permit p53 to bind to DNA to establish the cancer-killing cascade in tumor cells. IFN- $\alpha$ , IFN- $\beta$ , and IFN- $\gamma$  have been found to have significant roles and pathways to induce apoptosis (Clemens, 2003). In the past ten years, a number of reports have begun to relate interactions of interferons (IFNs) and p53. Because both the tumor suppressor p53 and the interferons exhibit antioncogenic activity, Takaoka et al. (2003) asked if there is a link between p53 and the interferons in oncogenesis. They examined the effects of IFN- $\alpha$  and IFN- $\beta$  on p53 expression and activity and the effects of p53 on the actions of IFN- $\alpha$  and IFN- $\beta$  and demonstrated that there is a link between the interferons (IFN- $\alpha$  and IFN- $\beta$ ) and p53 (Takaoka et al., 2003), a conclusion also made by others (Mecchia et al., 2000).

Takaoka et al. (2003) demonstrated that p53 can be induced by IFN- $\alpha$  and IFN- $\beta$ . Mouse embryo fibroblasts (MEF) and human hepatoma cancer cell lines HepG2 and HLE treated with IFN- $\beta$  showed an increase in the p53 protein.

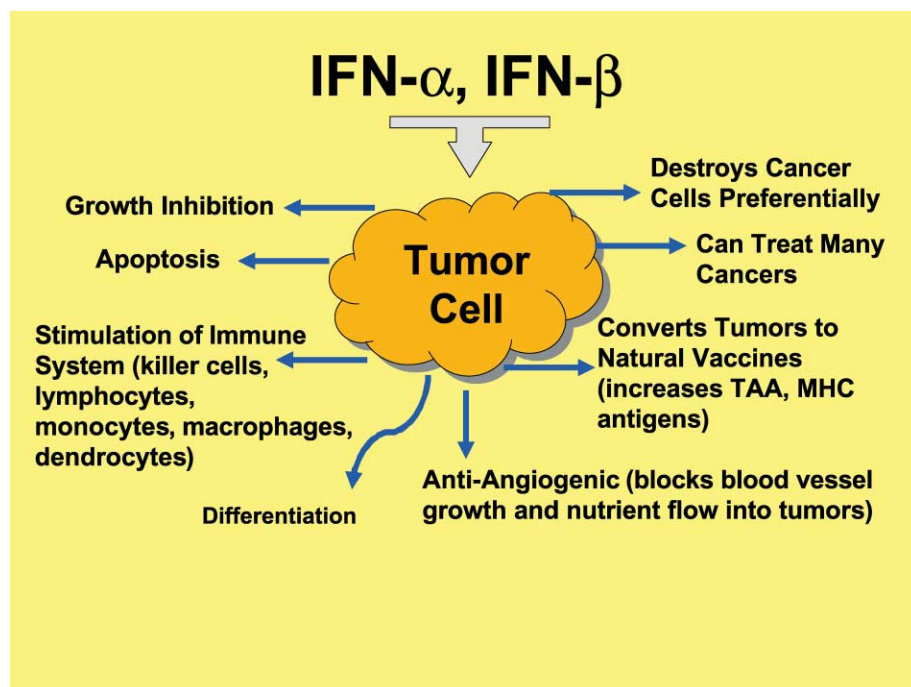
The p53 mRNA was induced about 3-fold with IFN- $\beta$  in MEF but not in MEF from IRF-9 $^{-/-}$  mice, consistent with the requirement for the intact IFN-activated transcription factor ISGF3 (Stat1, Stat2, and IRF-9) for induction. Two interferon-stimulated response elements (ISRE) were identified in the p53 gene. An anti-Stat2 antibody was used for chromatin immunoprecipitation from cells treated with IFN- $\beta$ ; both p53-ISREs were found. Therefore, IFN- $\beta$  induces transcriptional activation of the p53 gene through the ISREs. However, IFN- $\beta$  did not induce activation of p53, i.e., phosphorylation, nor induction of p53 target genes in MEF. Based on the general observations that interferons have greater growth-inhibitory activity on tumor rather than normal cells, Takaoka et al. examined cells containing the E6 human papilloma virus (HPV) oncogene. In cells expressing HPV E6, IFN- $\beta$  increased p53 mRNA and protein expression greater than in normal MEF. The authors examined colony formation of MEF transfected with E6 + Ras and MEF p53 $^{-/-}$  transfected with Ras as a function of IFN- $\beta$  concentration. IFN- $\beta$  reduced colonies of MEF with E6 + Ras about 80% while reducing colonies of MEF p53 $^{-/-}$  with Ras only about 10%. When they examined the effect of IFN- $\beta$  on apoptosis in X-ray-irradiated MEF transfected with the adenovirus E1A protein, they found that pretreatment of cells with IFN- $\beta$  prior to irradiation increased apoptosis from about 50% to 95%, but had little effect on nonirradiated cells. Studies with the chemotherapeutic DNA-damaging agent 5-FU similarly showed that IFN- $\beta$  (10,000 unit/ml) with 5-FU decreased the viability of HepG2 cells about 50% compared to cells treated with 5-FU alone, and only in cells with p53. All these observations suggest that antitumor activity of IFN- $\alpha$  and IFN- $\beta$  is mediated at least in part by p53.

To relate p53 to antiviral activity of

IFN- $\beta$ , Takaoka et al. examined changes in p53 in response to viral infection and IFN- $\beta$ . When cells were infected with vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), or herpes simplex virus (HSV), the quantity of p53 protein and p53 phosphorylation was increased and required an active ATM kinase that phosphorylates p53. In addition, some p53 target genes were induced by viral infection (*Mdm2*, *Puma*), but some other genes induced by DNA damage were not (*p21<sup>WAF1/Cip1</sup>*, *Noxa*). The results demonstrated that p53 is activated, but virus infection and DNA damage induced different target genes. As expected, large levels of IFN- $\beta$  were produced during the viral infection. Although on virus infection no increase in p53 mRNA was seen in MEF IFNAR1 $^{-/-}$  cells (cells lacking the receptor for IFN- $\alpha$  and IFN- $\beta$ ), phosphorylation of p53 did occur in MEF IFNAR1 $^{-/-}$  cells, indicating that activation of p53 is not dependent on IFN- $\beta$ . MEF infected with VSV strongly undergo apoptosis, but apoptosis was reduced in p53 $^{-/-}$  MEF and the virus yield was increased 30-fold. Of great import was a single in vivo experiment in mice. All p53 $^{-/-}$  mice infected with VSV died within 16 days while about 80% of wild-type mice survived. Furthermore, the p53 $^{-/-}$  mice had 100-fold higher titer of VSV in serum. These results allow several important conclusions: the activation of p53 and the induction of p53 are independent—IFN- $\beta$  only affects the induction, but does not contribute to its activation; different target genes are induced by p53 in response to DNA damage in comparison to viral infection—a result that means that induction of p53 genes can be modulated by other factors that are yet to be discovered; and p53 is a significant regulator of viral yield and ultimate fate of the host in an infection.

### Remarks

The data clearly support the authors'



**Figure 1.** Antitumor actions of interferons

The actions of IFN- $\alpha$  and IFN- $\beta$  that contribute to their antitumor activity are shown in this figure. These interferons act on the tumor cells, the tumor stroma and cells of the immune system that result in destruction of tumor cells and tumors. Interferons help identify cancer cells to the immune system by stimulating production of cell surface molecules such as MHC complex antigens, tumor-associated antigens (TAA), and costimulatory molecules. Interferon also activates cells of the immune system (cytotoxic lymphocytes, natural killer cells, macrophages, monocytes, and dendritic cells) that eliminate tumor cells throughout the body. A summary of these actions is shown in the figure.

conclusions: transcription of p53 is induced by IFN- $\alpha$  and IFN- $\beta$ ; the higher p53 levels enable increased apoptosis by IFN- $\alpha$  and IFN- $\beta$ ; p53<sup>-/-</sup> mice succumb to VSV infection more readily than wild-type mice. It would be anticipated that most Type I IFNs (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\epsilon$ , IFN- $\kappa$ , IFN- $\omega$ , IFN- $\delta$ , and IFN- $\tau$ ) will induce p53 similarly, but, because they often exhibit substantial differences in activity (Pestka et al., 2004), there might be significant variations. Takaoka et al. (2003) will undoubtedly stimulate many others to follow up in many avenues. Nevertheless, many investigators have concluded that other pathways link IFN- $\alpha$  and IFN- $\beta$  to apoptosis (Clemens, 2003), so these apparent discrepancies will need to be resolved.

#### **Impact on cancer and viral therapy**

Their results suggested that chemotherapeutics and irradiation should be tried in conjunction with interferons to minimize doses and side effects. This has been tried in clinical trials with spotty results. However, the fact that the levels of interferon Takaoka et al. (2003) used in some experiments were as high as 10,000 unit/ml makes it unlikely that their results could be simply translated to patients because of intolerable side effects. High levels of IFN- $\alpha$  are effective in advanced melanoma, yet patients often cannot tolerate the high doses (Kirkwood, 2002). Nevertheless, IFN- $\alpha$  and IFN- $\beta$  have the innate power to

accomplish this, but it will take new challenging initiatives and technologies (e.g., sustained delivery systems, eliminating side effects) to apply IFNs locally and minimize the debilitating side effects of systemic IFN (Lavoie et al., 2003). For example, new interferons have been developed that have higher activities than the standard ones in clinical use and could lessen side effects (Lavoie et al., 2003; Pestka, 1998, 1999). Furthermore, apoptosis is only one aspect of cancer therapy with interferon. The type I interferons exhibit a wide breadth of biological activities: antiviral, antiproliferative, stimulation of cytotoxic activity of many cells of the immune system (T cells, natural killer cells, monocytes, macrophages, dendritic cells), increased expression of tumor-associated surface antigens, stimulation of MHC class I antigens, induction and/or activation of proapoptotic genes and proteins (e.g., TRAIL, caspases, Bak and Bax, p53), repression of antiapoptotic genes (e.g., Bcl-2, IAP [inhibitor of apoptosis protein]), modulation of differentiation, and antiangiogenic activity (Figure 1). All these actions make interferon a most promising agent to treat various diseases, especially cancer and viral infections. The challenge is to be able to use the enormous power of the interferons without the debilitating side effects. Appropriate technology to deliver the interferons to tumors locally (Lavoie et

al., 2003; Mecchia et al., 2000) could overcome the problem of systemic side effects. Overall, it is highly likely that interferons will play a major role in the next generation of novel antitumor and antiviral therapies. The enormous progress in understanding how these molecules (interferons, p53) function, however, paves the way for their future use in combating many maladies of humanity.

Sidney Pestka<sup>1,2,3</sup>

<sup>1</sup>Department of Molecular Genetics, Microbiology and Immunology  
Robert Wood Johnson Medical School  
UMDNJ, 675 Hoes Lane  
Piscataway, New Jersey 08854

<sup>2</sup>PBL Biomedical Laboratories  
131 Ethel Road West  
Piscataway, New Jersey 08854

<sup>3</sup>Cancer Institute of New Jersey  
New Brunswick, New Jersey 08901  
E-mail: pestka@waksman.rutgers.edu

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