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SENSITIVITY TO LOW-DOSE RADIATION IN RADIOSENSITIVE "WASTED" MICE

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Abstract

Mice homozygous for the autosomal recessive wasted mutation (*wst/wst*) have abnormalities in T-lymphocytes and in the anterior motor neuron cells of the spinal cord, leading to sensitivity to low doses of ionizing radiation, hind limb paralysis, and immunodeficiency. This defect results in a failure to gain weight by 20 days and death at 28 days of age. The wasted mutation (previously mapped to mouse chromosome 2) is shown to be a 3-bp deletion in a T-cell-specific (and perhaps motor-neuron-specific) regulatory region (promoter) of the proliferating cell nuclear antigen (PCNA) gene on mouse chromosome 2. A regulatory element is also shown to be important in PCNA expression in T-lymphocytes and motor neuron cells affected by the 3-bp deletion in the PCNA promoter. The model is as follows: Absence of PCNA expression in the thymuses (and motor neurons) of wasted mice causes cellular apoptosis; this absence of expression is mediated by a positive transfactor that can bind to the wild-type but not the wasted mutant PCNA promoter; the bound protein induces late expression of PCNA in T-lymphocytes and prevents onset of radiation sensitivity in the cells.

Wasted mice were first described by Schultz *et al.* (1) as mutants showing evidence of neurologic abnormalities as early as 20 d after birth. Standard features of the disorder include tremulousness, immunodeficiency, and faulty repair of DNA damage induced by ionizing radiation, especially in lymphocytes (2). Recent studies in our laboratory have defined the molecular lesion in the wasted mouse model as a 3-bp deletional mutation in the promoter region of the PCNA gene. Our model is as follows: Absence of PCNA expression in the thymuses (and motor neurons) of wasted mice causes cellular apoptosis; this absence of expression is mediated by a positive transfactor that can bind to the wild-type but not the wasted mutant PCNA promoter; and induces late expression of PCNA in T-lymphocytes.

Table 1 summarizes results of published (2B6) and unpublished work examining the effects of low-dose (1/2 Gy) g-ray exposure on a variety of cellular and molecular responses to radiation. Lymphocytes from wasted mice show higher spontaneous and radiation-induced cell depletion rates from thymic and splenic tissues than control litter mates. In addition, normal cells arrest transcription for the first 10 min to 1 h following radiation exposure (3), but cells from wasted mice enhance transcriptional activity two-fold following 1/3 Gy exposures. Because wasted mice are defective in PCNA expression, it is possible that PCNA plays a role in the regulation of radiation-mediated transcriptional inhibition. While transcription is enhanced in wasted mice following radiation exposure, specific induction of at least two known radiation-modulated genes (*c-fos*, VL30) is the same as that found in control mice. Differential display (5) analysis (Table 2) of a total of 635 bands using 13 different primer pairs compared thymus mRNA from untreated and irradiated (3 Gy g-rays) *wst/wst*, *wst* littermates and age-matched control BCF₁ mice. It should be noted that

RNA samples from untreated wasted mice amplified poorly, giving only five identifiable bands in repeated experiments; the reason is unknown, but this has been noted previously (6). Of a total of 635 bands, 20 (or 3%) were affected by total body g-irradiation of mice, and of these three were differentially affected by radiation in *wst/wst* mice compared with their normal littermates. In addition, we observed several differences between BCF and *wst* mice that appear to be of strain-specific and not related either to the *wst* trait or to radiation exposure.

These results implicate PCNA as an important functional component of the thymic cellular response to ionizing radiation. This is not surprising in light of the fact that PCNA contributes to cell cycle regulation and thymic lymphocytes undergo multiple rounds of cell division. Motor neuron cells of wasted mice that are also affected by the PCNA lesion do not divide, indicating that PCNA may make other functional contributions.

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Table 1

Radiation-Sensitivity of Lymphocytes of Wasted (*wst/wst*) Mice¹

Feature	Untreated	Irradiated
Cell death	↑	↑↑
Apoptosis	↑	↑↑
Transcription	control levels	↑
<i>c-fos</i> induction	control levels	control levels
VL30 induction	control levels	control levels
Mitogen-induced cell cycle distribution	normal	normal

¹Comparisons are made relative to *wst/+* and *+/+* littermates.

Table 2

Analysis of Differential Display Bands from Irradiated Mice

cDNA Category	Number of Bands in Each Category			Total no. of bands ₁
	Wasted Mice	Wasted Littermates	BCF1 Mice	
Upregulated upon irradiation	4	4	3	7
Downregulated upon irradiation	6	4	5	13
Strain-specific upregulated	3	3	0	3
Strain-specific downregulated	8	8	0	8
Total number of cDNA with change in expression pattern				31
Total number of cDNA observed with 13 primer pairs				635

₁Total number is less than the sum of values in preceding cells of the table because some bands overlap between samples.