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The mouse ERG before and after light damage is independent of p53

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Abstract. Death of retinal photoreceptors by apoptosis is observed under many physiological and pathological conditions such as histogenesis, retinal dystrophies and light-induced photoreceptor degeneration. To date, little is known about regulatory mechanisms for apoptosis in the retina. The tumor suppressor gene *p53* is a regulator of apoptosis in a number of systems, however, p53-independent apoptosis has also been described. We have therefore investigated whether the lack of p53 influences the dark-adapted ERG in C57BL/6 $p53^{-/-}$ mice compared to $p53^{+/+}$ control littermates under physiological (regular light-dark cycle) conditions. We also recorded ERGs at 12 to 14 h in darkness following diffuse bright light exposure to 8′000 or 15’000 lux for 2 h. ERG analysis over a range of 6 logarithmic units of light intensity revealed normal and virtually identical a-, b-, c-waves and oscillatory potentials in dark-adapted $p53^{+/+}$ and $p53^{-/-}$ mice. After exposure to diffuse white fluorescent light strong decreases of all ERG components were found to be very similar in both genotypes. These data support the notion that the p53 protein is neither essential for normal retinal function nor for processes involved in light-induced depression of the ERG in mice.

Key words: apoptosis, electroretinogram, retinal light damage, p53, transgenic mice

Introduction

The electroretinogram (ERG) is a potent tool for the investigation of the functional state of the retina. Therefore, it has been used frequently to assess retinal function in the rapidly growing field of transgenic animal models for retinal degeneration [1-4]. Cell death by apoptosis is observed *in vivo* and *in vitro*. It involves many biological and medical disciplines [5, 6], and numerous stimuli and conditions can induce this type of cell death. In the retina, apoptosis is the fate of ‘surplus’ photoreceptors during histogenesis [7], in several animal models of retinitis pigmentosa (RP) [8, 9], in human RP [10], and in light-induced photoreceptor degeneration [11, 12]. The tumor suppres-

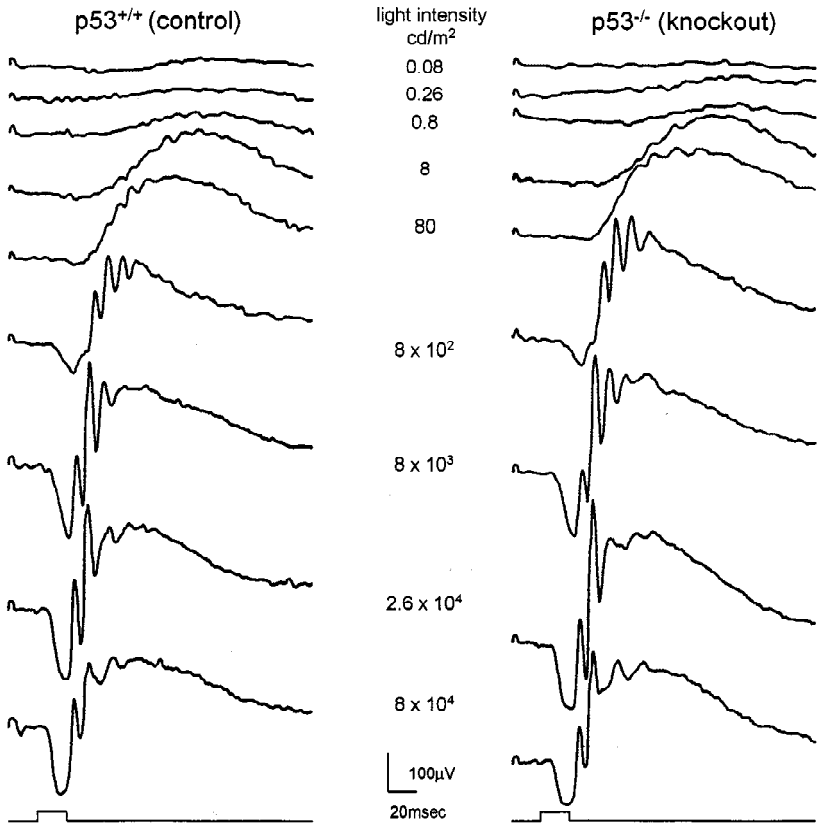

Figure 1. ERG a- and b-waves recorded from dark-adapted p53 control and p53 knockout mice without bright light exposure. Here and in the subsequent figures traces represent the average of 4 signals and the lowermost trace represents with upward deflection the light stimulus of 20 msec. Bandpass 0.3 – 1000 Hz.

sor gene *p53* is an important regulator of apoptosis in a variety of systems and tissues [13]. However, p53-dependent and -independent apoptosis has been observed [14-16]. In a previous study, we have therefore investigated whether the absence of functional p53 influences the rate of apoptotic cell death in a model of light-induced photoreceptor degeneration [17]. In the present study, we additionally addressed in detail the functional state of retinas lacking p53 under physiological and pathological conditions [18, 19]. First, we investigated whether the lack of p53 during development of the retina influences the ERG of adult mice kept in a regular 12:12 light-dark cycle. Second, we induced photoreceptor degeneration in $p53^{-/-}$ and $p53^{+/+}$ mice by exposure to diffuse bright light and compared the ERG responses. Our data suggest that

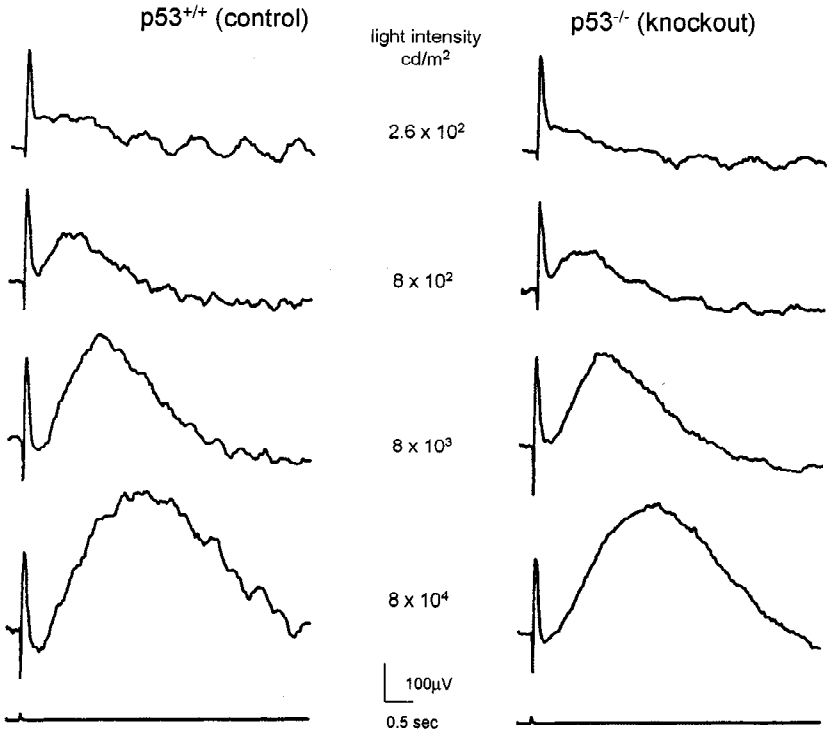

Figure 2. ERG c-waves recorded from dark-adapted p53 control and p53 knockout mice without bright light exposure. Bandpass 0.03 – 1000 Hz.

p53 is neither essential for normal retinal function nor involved in processes following light-induced photoreceptor degeneration.

Materials and methods

All procedures were in accordance with the regulations of the Cantonal Veterinary Authority of Zurich and with the ARVO resolution for the Care and Use of Animals in Ophthalmic and Vision Research. Mice investigated (n=24, 6 per experimental condition and genotype) were 6 to 12 weeks old, and *p53* knockouts were bred from heterozygous matings on a C57BL/6 background. Their genotype was determined by PCR analysis of genomic DNA. All animals were maintained in a 12:12 h dark-light cycle (light on at 6 am) with 10-20 lux within the cages. Twelve mice underwent ERG recording at the same time of the day in dark adaptation without any bright light exposure. The other 12 animals were exposed to 8000 or 15’000 lux of diffuse, cool,

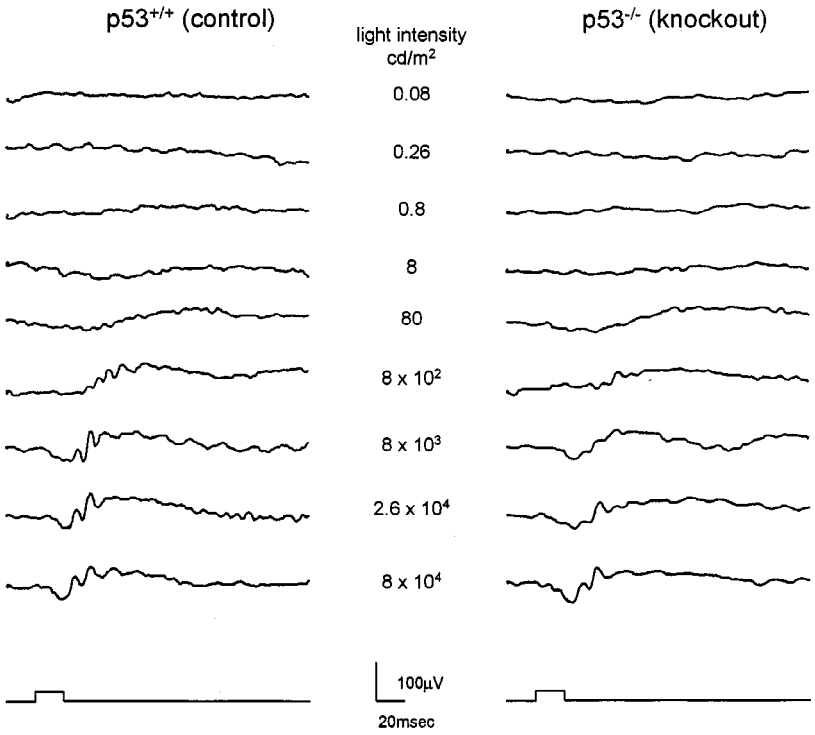

Figure 3. ERG a- and b-waves recorded from dark-adapted p53 control and p53 knockout mice 12 h after bright light exposure.

white fluorescent light for 2 h, and ERGs were recorded at 12 to 14 h in darkness following light exposure.

Anesthesia was performed with a single intraperitoneal injection of xylazine 20 μg/g and ketamine 40 μg/g, and the anesthetized animal was placed on a heating pad (37 °C). DC-wick-electrodes [20] were placed on the dilated (tropicamide, Mydriaticum Dispersa$^{(R)}$, Ciba Vision, Niederwangen, Switzerland) left eye and, as a reference electrode, in the mouth. A thin platinum ground-electrode was fixed subcutaneously in the tail. All manipulations were performed under very dim red light with an operating microscope, and the position of the corneal electrode was monitored with an infrared camera. Ganzfeld electroretinograms recorded from dark-adapted mice were elicited by pulses of white light of 20 msec duration from a halogen source (Intralux, Volpi, Urdorf, Switzerland) and were presented in increasing order over a range of 6 logarithmic units of light intensity ($8x10^{-2}$ - $8x10^{4}$ cd/m^2). Original traces of 4 consecutively averaged responses were plotted on a 2-channel chart recorder (Kipp & Zonen, Recom Electronic AG, Horgen,

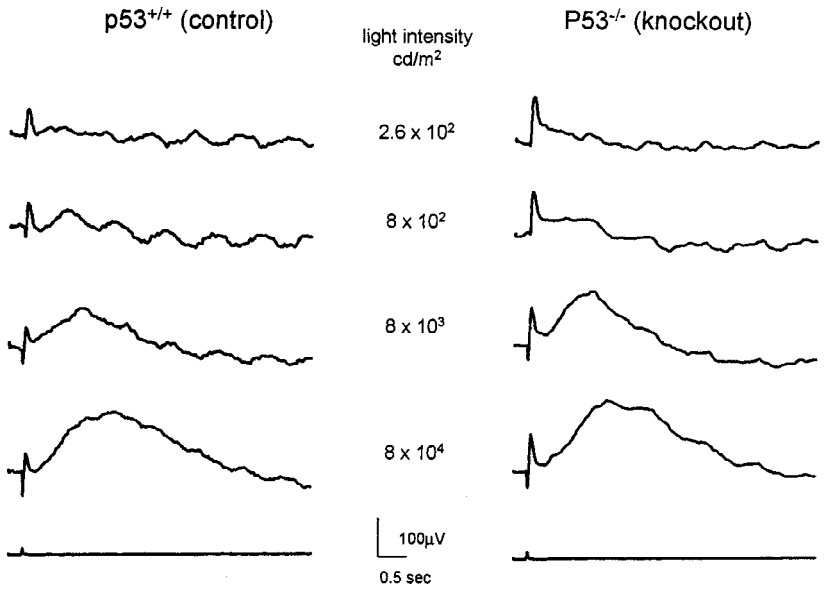

Figure 4. ERG c-waves recorded from dark-adapted p53 control and p53 knockout mice 12 h after bright light exposure.

Switzerland) and digitally stored on a personal computer for off-line analysis [21]. Analogues filters (Krohn Hite 3750, Avon, MA) provided a bandpass of 0.3-1000 Hz for the a- and b-waves, 60-300 Hz for the OPs, and 0.03-1000 Hz for the c-waves. After ERG recordings following a rigid protocol, the anesthetized animals were killed by cervical dislocation, the left eye was processed for histology, and the fellow eye for TdT-mediated dUTP nick-end labeling (TUNEL staining) [17].

Results

Both $p53^{-/-}$ mice and $p53^{+/+}$ control littermates taken from a regular 12:12 light-dark cycle and dark adapted for 36 h displayed comparable ERG recordings: a-waves, b-waves as well as c-waves (Figures 1 and 2) and oscillatory potentials (data not shown) were virtually identical regarding time course, amplitude and configuration. This was not only true for single stimulus intensities, but also for the V/logI function over 6 log units of light intensity, where data from controls and knockouts were indistinguishable. (Figures 5,6,7).

After exposure to bright light for 2 h and allowing a 12-14 h post-exposure time, the ERGs (Figures 3 and 4) revealed marked and comparable decreases in amplitudes and raised thresholds in both the *p53* wild-type and in the *p53*

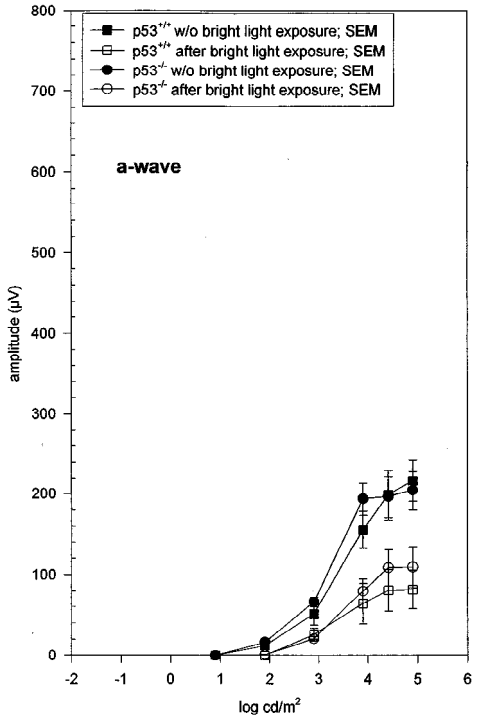

Figure 5. V/logI function of the a-wave from p53 control and p53 knockout mice without (filled symbols) and after (empty symbols) bright light exposure. Here and in the subsequent figures: n = 6 animals per group. Error bars represent ± SEM in this and subsequent figures.

knockout mice (Figures 5,6,7). The ERG alterations observed were in the same range for both intensities (8'000 and 15'000 lux) used during light exposure for 2 h. These changes in ERG components at all stimulus intensities indicate a severe functional impairment due to light damage in both genotypes. The ERG data shown here correlate well with the histological and TUNEL findings presented by Marti et al. [7]. Morphologically, the outer retina of both the *p53* wild-type and the *p53* knockout mouse revealed massive apoptosis of photoreceptor nuclei and disrupted rod outer and inner segments at 12-14 h after light exposure, whereas controls showed regular retinal morphology in both genotypes [17]. In accordance to morphological findings, TUNEL showed distinct signals in the outer nuclear layer of both the *p*53 wild-type and the *p*53 knockout mouse at 12-14 h after light exposure whereas the retinas of controls were devoid of TUNEL signals in both genotypes [17].

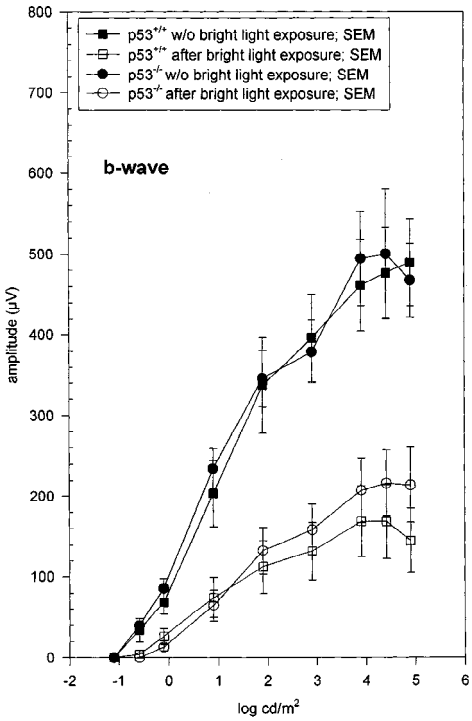

Figure 6. V/logI function of the b-wave from p53 control and p53 knockout mice without (filled symbols) and after (empty symbols) bright light exposure.

Discussion

p53 is not essential for normal retinal function

Comparison of full-field ERG recordings from control and *p*53 knockout mice indicate that the p53 protein is not essential for normal retinal function. The cornea negative a-wave, generated by photoreceptors, the b-wave representing inner retinal function as well as the c-wave – an expression of a photoreceptor/RPE interaction - from $p53^{+/+}$ and $p53^{-/-}$ mice, respectively, were virtually identical. This observation is evident in original waveforms as well as in the corresponding amplitude/intensity functions. The latter revealed that the electrophysiologically assessed sensitivity of the retina over a range of 6 log units of light intensity was not different in p53 deficient mice compared to control mice. Therefore we conclude that the p53 protein is not essential for normal electrophysiological function of the retina. Furthermore, these findings show that the lack of functional p53 during development of the retina does not alter retinal electrophysiology.

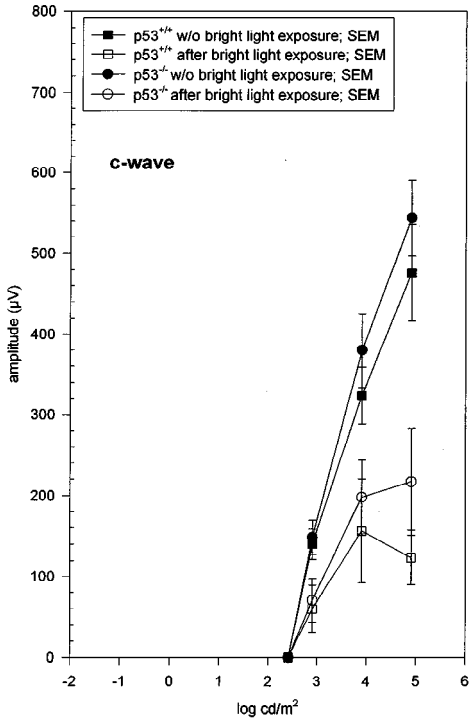

Figure 7. V/logI function of the c-wave from p53 control and p53 knockout mice without (filled symbols) and after (empty symbols) bright light exposure.

Light-induced ERG changes are independent of p53

Exposure to diffuse bright light induced marked and comparable decreases in the ERG parameters in both the control and in the *p53* knockout animals, indicating a severe functional impairment of the retina. Therefore, the absence of p53 did not alter the electrophysiological response of the mouse retina in light-induced photoreceptor degeneration. These data complement and corroborate the morphological, histochemical and biochemical findings from Marti et al. [17] which show that light-induced photoreceptor apoptosis in the mouse retina is independent of functional p53.

In conclusion, apoptosis in the retina is independent of functional p53 during development and after light-induced photoreceptor degeneration. In addition, measurement of the ERG response is a sensitive and important tool to monitor functional changes observed after light-induced photoreceptor degeneration. It would therefore be interesting to monitor, in future studies, a correlation of the extent of light-induced lesions with the magnitude of ERG changes.

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