The American Journal of Pathology, Vol. 185, No. 1, January 2015

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

CELL INJURY, REPAIR, AGING, AND APOPTOSIS

The Patched 1 Tumor-Suppressor Gene Protects the Mouse Lens from Spontaneous and Radiation-Induced Cataract

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Accepted for publication September 4, 2014.

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Age-related cataract is the most common cause of visual impairment. Moreover, traumatic cataracts form after injury to the eye, including radiation damage. We report herein that sonic hedgehog (Shh) signaling plays a key role in cataract development and in normal lens response to radiation injury. Mice heterozygous for Patched 1 (*Ptch1*), the Shh receptor and negative regulator of the pathway, develop spontaneous cataract and are highly susceptible to cataract induction by exposure to ionizing radiation in early postnatal age, when lens epithelial cells undergo rapid expansion in the lens epithelium. Neonatally irradiated and control $Ptch1^{+/-}$ mice were compared for markers of progenitors, Shh pathway activation, and epithelial-to-mesenchymal transition (EMT). Molecular analyses showed increased expression of the EMT-related transforming growth factor β/Smad signaling pathway in the neonatally irradiated lens, and up-regulation of mesenchymal markers Zeb1 and Vim. We further show a link between proliferation and the stemness property of lens epithelial cells, controlled by Shh. Our results suggest that Shh and transforming growth factor β signaling cooperate to promote *Ptch1*-associated cataract development by activating EMT, and that the nanog marker of pluripotent cells may act as the primary transcription factor on which both signaling pathways converge after damage. These findings highlight a novel function of Shh signaling unrelated to cancer and provide a new animal model to investigate the molecular pathogenesis of cataract formation. *(Am J Pathol 2015, 185: 1–11; http://dx.doi.org/10.1016/j.ajpath.2014.09.019)*

Cataract is the most frequent cause of blindness worldwide.[1] Development of cataract in adults is related to normal aging, but other factors are also involved, such as diabetes, smoking, and alcohol use, as well as exposure to UVB and ionizing radiations.[2] Cataract is also one of the most important causes of avoidable (preventable and treatable) childhood blindness,[3] and often develops as a complication of radiotherapy.[4] However, the exact mechanisms of radiogenic cataract are not entirely understood.

Inherited mutations of the Patched 1 (*Ptch1*) gene have been identified as responsible for Gorlin syndrome.[5] Patients with this hereditary condition are hypersensitive to radiation and prone to develop multiple skin cancers, specifically basal cell carcinomas,[6] occurring with greater incidence in portals of radiotherapy.[7] They are also at risk for developing medulloblastoma, a specific type of brain tumor. Among many minor criteria for diagnosis, ophthalmic abnormalities, including cataract, occur in 26% of patients.[8,9]

Mice in which one copy of *Ptch1* is inactivated are characterized by activation of the sonic hedgehog (Shh) pathway and show increased susceptibility to spontaneous and radiation-induced tumors, providing an ideal *in vivo* model for studying the typical pathological conditions associated with Gorlin syndrome.[10–12] In addition to the well-known role of hedgehog signaling in cancer, Shh signals direct cell proliferation, cell fate determination, epithelial-to-mesenchymal transition (EMT), and the rearrangement of cells by motility and adhesion changes during embryogenesis, remaining largely expressed in the stem

Supported in part by the Unit of Radiation Biology and Human Health, l'Energia e lo Sviluppo Economico Sostenibile, Intramural Program (M.M. and A.S.).

I.D.S., B.T., and P.G. contributed equally to this work.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2014.09.019

cell compartment of adult tissues.[13] The vertebrate ocular lens, composed of epithelial and fiber cells, grows throughout life, implying the existence of a lens stem cell compartment. DNA labeling studies showed that most new lens cells arise in the germinative zone, a narrow cellular region that rings the lens epithelium toward the periphery of the anterior lens surface.[14,15] After division, these cells withdraw from the cell cycle and terminally differentiate into fiber cells. Despite findings showing a role of Shh in mouse lens development[16] and in lens regeneration of the adult newt,[17] much remains to be understood about the role of this pathway in postnatal lens growth and its possible correlation with cataract development.

Herein, we show a specific role for the *Ptch1* gene in the maintenance of lens integrity with or without exogenous damage, and validate the *Ptch1*$^{+/-}$ model as a tool to understand the molecular mechanism of cataract development. Altogether, our results support the hypothesis that Shh and transforming growth factor β (TGF-β) signaling pathways synergize with radiation damage to induce EMT, culminating in anterior subcapsular cataract (ASC) development. We also outline a role of the nanog stemness gene in *Ptch1*-associated radiogenic cataract.

Materials and Methods

Mice

Mice lacking one *Ptch1* allele (*Ptch1*$^{+/neo6/7}$; named *Ptch1*$^{+/-}$ throughout the text) on CD1 background were bred and genotyped as described.[10] Care of experimental animals was in accordance with the Italian legislation on animal experimentation. Experimental protocols were reviewed by the Institutional Animal Care and Use Committee.

Mice Irradiation and Monitoring

Ptch1$^{+/-}$ and wild-type (WT) littermates of both sexes were irradiated using a Gilardoni CHF 320 G X-ray generator (Gilardoni, Mandello del Lario, Italy), as described.[12] Animals were exposed to a single dose of 3 Gy at different postnatal (P) days (P2, P10, P56). Additional groups were left untreated as controls. Experimental groups are summarized in Table 1. [T1]

Mice were observed daily throughout their life span. The time of cataract onset (unilateral and bilateral) (Table 1) was recorded. On decline of health, mice were sacrificed and necropsied.

Histological, IHC, and Morphometric Analysis

Normal and cataract-bearing eyes were processed for histological analysis by standard techniques at the end of their lifetime or when bilateral cataract became evident; eyes were also collected from mice sacrificed before termination of the experiment. Eye sections (2 μm thick) were cut in a plane perpendicular to the anteroposterior eye axis and stained with hematoxylin and eosin. Immunohistochemistry (IHC) was performed on the following groups: i) eyes from satellite groups ($n = 6$ per genotype) irradiated at P2 and sacrificed at short-term after irradiation (ie, 0.5 hours for analysis of γ-H2AX foci (a biomarker of DNA double-strand breaks), 4.5 hours for detection of p53 and p21 immunoreactivity, and 6 hours for apoptotic analysis by activated caspase-3 antibody); ii) eyes from unirradiated mice at P2, P4, P6, and P10 or P2-irradiated mice sacrificed at different times after irradiation (P4, P6, and P10) for Ki-67 proliferation analysis and nestin immunoreactivity; and iii) fully developed cataracts. The following antibodies were used: Ki-67 (NCL-Ki67p; polyclonal; dilution 1:800; Novocastra Laboratories, Newcastle, UK); γ-H2AX (05-636; monoclonal; dilution 1:200; Upstate Biotechnology Inc., Lake Placid, NY); cleaved caspase-3 (9661; polyclonal; dilution 1:100; Cell Signaling Technology, Inc., Danvers, MA); E-cadherin (3195; polyclonal; dilution 1:200; Cell Signaling Technology, Inc.); N-cadherin (13116; polyclonal; dilution 1:200; Cell Signaling Technology, Inc.); anti-nestin (ab81755; polyclonal; dilution 1:300; Abcam, Cambridge, UK); α-smooth muscle actin (A2547;

Table 1 Incidence of Cataract in *Ptch1*$^{+/-}$ and *Ptch1*$^{+/+}$ Mice Irradiated at 2, 10, and 56 Days of Age with 3 Gy of X-Rays or Left Unirradiated

Treatment	Age, days	Mouse line	No. of mice	Unilateral cataract, % (no./total)	Bilateral cataract, % (no./total)	Median latency (weeks)
3 Gy	2	*Ptch1*$^{+/-}$	42	45.2 (19/42)*†	14.3 (6/42)‡	6.9
		Ptch1$^{+/+}$	48	8.3 (4/48)§	2.08 (1/48)	9.5
3 Gy	10	*Ptch1*$^{+/-}$	33	0	0	—
		Ptch1$^{+/+}$	47	0	0	—
3 Gy	56	*Ptch1*$^{+/-}$	44	2.27 (1/44)	0	7
		Ptch1$^{+/+}$	39	2.56 (1/39)	0	32
0 Gy	—	*Ptch1*$^{+/-}$	34	8.8 (3/34)	5.88 (2/34)	34
		Ptch1$^{+/+}$	53	0	0	—

All *P* values were calculated using Fisher's exact test.
*$P < 0.001$ between unilateral cataract P2 irradiated *Ptch1*$^{+/-}$ mice and unirradiated *Ptch1*$^{+/-}$ mice.
†$P < 0.0001$ between unilateral cataract P2 irradiated *Ptch1*$^{+/-}$ and *Ptch1*$^{+/+}$ mice.
‡$P < 0.05$ between bilateral cataract P2 irradiated *Ptch1*$^{+/-}$ and *Ptch1*$^{+/+}$ mice.
§$P < 0.05$ between unilateral cataract P2 irradiated *Ptch1*$^{+/+}$ mice and unirradiated *Ptch1*$^{+/+}$ mice.
Ptch1, Patched1.

FLA 5.2.0 DTD ■ AJPA1879_proof ■ 19 November 2014 ■ 7:24 pm ■ EO: AJP14_0357