Correspondence

Dear Editor:

With great interest we read the article by Dr. Kimura and collegues.[1] The authors introduced interesting results of surgery that testify for anatomic closure that may be achieved only by creating a posterior vitreous detachment without internal limiting membrane (ILM) removal. It is evident that contraction of the proliferative cells causes the macular hole enlargement.

However, an explanation of illustrations is not entirely correct, and the authors do not exactly explain the role of vitreomacular traction in pathogenesis of macular holes. For example, in Figure 1b in Kimura et al's article, the partial detached posterior vitreous membrane is disposing in the form of a bow. But if it is the cause of enlargement of macular holes, it should be tight as a string between the points of its attachment (foveola and optic nerve head).

More precisely, it is evident at early stages of macular hole formation. As is shown in the optical coherence tomography image of the prehole (Figure 1), main tractional forces are directed to the vitreous body center instead of along posterior hyaloid membrane.

In our experience, vitreous contraction causes vitreomacular tractions that put out a fragment of inner retina, followed by fluid accumulation in the retina, as considered by Tornambe.[2]

Our opinion of the pathogenesis of macular holes differs a little from the view of Kimura and colleagues. Nevertheless, it does not contradict the statement of the authors that in newly formed macular holes the ILM still has no significant tension and its removal is not required.

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Reply

Dear Editor:

The illustration has some problems, as Dr. Alpatov pointed out. In that Figure, we meant to show the action point and the responsible tissue of traction force, not the direction of it. I agree that the traction force from partially detached posterior vitreous membrane mainly has the vector toward the center of the vitreous and the arrows should be omitted in Figure 1b.

We discussed the preventing factor of macular hole closure, not the pathogenesis. A force that does not appear to be strong enough to enlarge the hole may be strong enough to prevent closure of it.

Tomambe described an interesting theory that the fluid accumulation in the retina around the macular hole after the inner retinal defect prevents the closure, so that separation of the retinal surface from vitreous liquid is needed to close the hole.[1] We experienced failure of macular hole closure with an accidentally small amount of gas, though it was enough to separate the retinal surface and the vitreous. This indicates that some pushing force to the retinal pigment epithelium is necessary.

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Dear Editor:

We congratulate Aiello et al[1] for their very insightful article on the evolving guidelines for intravitreous injections. The techniques highlighted in their article will hopefully help reduce the incidence of endophthalmitis during a time in which the number of intravitreal injections is increasing.

We suggest an additional maneuver that may help further reduce the incidence of endophthalmitis. We prepare the eye according to the guidelines outlined in the article by Aiello et al. Briefly, after dilation, a topical anesthetic is applied to the globe, followed by povidone–iodine (5%) to the eyelids, eyelashes, and ocular surface. A lid speculum is placed, and additional povidone–iodine is placed on the globe. We then use a sterile cotton-tipped applicator to displace the overlying conjunctiva away from the scleral injection site. The injection is given, and the cotton-tipped applicator and displaced conjunctiva are immediately rolled back over the scleral injection site (in a manner similar to the technique we use during 25-gauge vitrectomy to create a discontinuity between conjunctival and scleral entries).[2] This technique may help decrease the number of organisms that directly enter the globe after injection as well as reduce the possibility

of an external vitreous wick, a factor implicated in the development of endophthalmitis.[3,4]

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Reply

Dear Editor:

On behalf of the expert panel, we thank Garg and Recchia for their interest in the evolving guidelines for intravitreal injections.[1] They suggest using a cotton-tipped applicator to displace the conjunctiva, which immediately after intravitreal injection is rolled back over the scleral injection site to create a discontinuity between conjunctival and scleral entries, as has been reported for use during 25-gauge vitrectomy.[2] Although this technique may help to reduce the risk of endophthalmitis, this additional maneuver may also displace organisms into the injection field and might, therefore, be associated with an increased risk of endophthalmitis. Because there are no scientific data to guide the ophthalmic community, the usefulness of conjunctival displacement in decreasing the risk of endophthalmitis is unknown.

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Dear Editor:

We point out an error and important omission in the otherwise excellent review by Abramson and Schefler[1] on retinoblastoma in a recent issue of *Retina*. On page 830, Abramson and Schefler discuss animal models for retinoblastoma and state that the model developed by Zhang et al[2] in *Cell Cycle* (July 2004) is the "first description of a heritable model of retinoblastoma." The first heritable model was reported in 1990 in which large T antigen, a protein that binds and inactivates RB protein family members, was expressed in mouse retina.[3] Abramson and Schefler likely meant the first inheritable "knockout" model of retinoblastoma (i.e., where RB gene deletion is used), but this is also inaccurate because our group reported the first inheritable knockout model[4] (the article as well as commentaries in *Cancer Cell*, *Nature Review of Cancer*, and *Journal of the National Cancer Institute* at the time can be downloaded from our Web site: http://vsr-p.uhnres.utoronto.ca/Bremner.html).

Our article (Chen et al[4]), featured on the cover of *Cancer Cell*, was submitted on December 20, 2003, accepted on May 18, 2004, and published in the June 14th issue. The article by Zhang et al in *Cell Cycle* was submitted on May 26, 2004, accepted on June 3, 2004, and published in the July issue. Abramson and Schefler did not discuss our report or the excellent work of MacPherson et al[5], whose article was published in the July 2004 issue of *Genes and Development*. MacPherson et al showed that deleting RB and the third member of the family, p130, also leads to retinoblastoma in the mouse.

Abramson and Schefler may have been misled by the inaccurate title of the article by Zhang et al. The erroneous claim to be "The First Knockout Model of Retinoblastoma" was unfortunate in view of the fact that we shared our in press article with Zhang et al on May 21, 2004, before their submission to *Cell Cycle*. They did not reference our work.

In addition to providing an inheritable knockout model of retinoblastoma, our study made several important advances. In the absence of RB or RB/p107, there is no increase in the number of progenitors (a

result that was confirmed by MacPherson et al), but all differentiating cells fail to exit the cell cycle. Ectopically dividing RB/p107–deficient differentiating rod, cone, bipolar, and ganglion cells undergo apoptosis; however, amacrine, horizontal, and Müller cells survive, and most of these cells eventually exit the cell cycle in concert with terminal differentiation. Sporadic retinoblastoma emerges in ≈60% of eyes, and nascent tumors express transcription factors that drive amacrine cell genesis (NeuroD and Math3), lack markers of other differentiating cell types (e.g., Crx, a photoreceptor marker), and are also devoid of the progenitor marker Chx10. These data indicate that RB does not modulate the cell cycle in progenitors but is critical for cell cycle exit in differentiating cells and pinpoint the naturally death-resistant differentiating RB/p107–deficient amacrine cell as the origin of mouse retinoblastoma. Given that RB is required for cell cycle exit in every differentiating mouse retinal cell type, it seems likely that this function will be conserved in the human retina. We suggest, therefore, that human retinoblastoma may also arise from an ectopically dividing differentiating cell type, rather than a progenitor or stem cell.

The fact that tumors arise from cells that are intrinsically death resistant implies that post-RB mutations seen in human retinoblastoma may not be required to overcome apoptosis, as previously hypothesized.[6] Instead, our data suggest that these mutations are required to overcome growth arrest when ectopically dividing cells terminally differentiate. The natural defense to RB loss in the retina appears to be terminal differentiation, not apoptosis. This is unfortunate because it provides ectopically dividing cells time to accumulate new mutations that prevent differentiation and permit neoplastic transformation. However, now that we know the cell of origin's strength, it will be critical to understand the underlying mechanism. The secrets therein may provide opportunities to develop novel directed therapies that eliminate RB-deficient cell types before they develop more malevolent characteristics.

In summary, our work pinpointed ectopically dividing differentiating cells rather than progenitors or stem cells as the cell of origin of retinoblastoma, provided a new explanation for the post-RB mutations seen in human retinoblastoma, presented a comprehensive analysis of the cell-specific effects of deleting RB and its relative p107, and described the first inheritable knockout model of retinoblastoma (for recent reviews see Dyer and Bremner[7] and Bremner et al[8]). Mention of these together with the complementary findings of MacPherson et al would have enhanced the excellent review by Abramson and Schefler.

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Reply

Dear Editor:

We thank Bremner et al for the nice comments about our recent review in *Retina*[1] and for identifying two corrections. They are correct that the article by Zhang et al[2] describes the first "knockout" mouse model and not the first "heritable" model, which was a transgenic oncogene model developed many years earlier. The second correction that Bremner et al identified was the omission of their article (Chen et al[3]) and a similar report by MacPherson et al[4] describing knockout mouse models of retinoblastoma that complement the data reported by Zhang et al. Our review covered a broad range of topics related to retinoblas-

toma with nearly 120 references, and the omission of those two references was in no way a reflection of their excellent work. Their pioneering work is an important contribution to the field, and we apologize to them and many other fine investigators for not including their work.

Beyond these two corrections, Bremner et al challenge the claim by Zhang et al that the two mouse models of retinoblastoma that they describe (*Chx10-Cre;Rb$^{Lox/-}$; p107$^{-/-}$* and *Chx10-Cre;Rb$^{Lox/-}$; p53$^{Lox/-}$; p107$^{-/-}$*) were the first knockout mouse models. As evidence for this challenge, Bremner et al cite the submission, acceptance, and publication dates for their article and those for the article by Zhang et al. They have raised an important question, and we will try to explain what/why we did.

Each of the three articles quoted by Bremner et al was published in 2004 in June and July: Chen et al, June; and Zhang et al and MacPherson et al, July. That would make the article by Chen et al the "first."

An electronic version of the article by MacPherson et al and of the article by Zhang et al appeared earlier than any of the published articles. By that standard, the article by MacPherson et al or Zhang et al was "first."

The article by Chen et al was submitted on December 20, 2003, while that of Zhang et al was submitted on May 26, 2004. The article by Zhang et al was accepted for publication within 1 week (June 3, 2004), which is a credit to their work. The article by Chen et al was accepted on May 18, 2004, which was ≈2 weeks before the article by Zhang et al, but it required revisions. Using dates of submission and acceptance, the article by Chen et al was then "first."

To further complicate the matter, Zhang et al mentioned that the breeding for their animals actually began in December 2002. That would imply that they were the "first" to create the model.

Depending on your definition, any of these three superb articles were the "first." All journals, however, whether electronic or hard copy, must go through the same process to make their articles available to the public by publishing the citation and abstract on Pub MED. Most scientists would use the Pub MED ID number as the most objective measure of determining "when" an article is published and therefore who was "first." The Pub MED numbers for these articles are as follows: Zhang et al, Pub MED ID: 15190215; Chen et al, Pub MED ID: 15193257; and MacPherson et al, Pub MED ID: 15231717.

The respected journal *Cell Cycle* accepted the designation of "The First Knockout Model of Retinoblastoma" for the article by Zhang et al, and in deference to them, we did too. The title of the article by Chen et al was "Cell-Specific Effects of RB or RB/p107 Loss on Retinal Development Implicate an Intrinsically Death-Resistant Cell-of-Origin in Retinoblastoma." On the basis of the Pub MED citation and title given to the article by *Cell Cycle*, we thought it appropriate to state that their article represented the first knockout model of retinoblastoma.

More important to us as clinicians are the elegance, sophistication, and ingenuity of each of these groups in the design of their experiments and high quality of their research. We sincerely believe that their collective input will help humankind and the patients we treat every day.

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