Lipofuscin Turnover

A recent article in IOVS by Katz et al. reported the reversible accumulation of lipofuscin-like inclusions in the retinal pigment epithelium of rats given a single intravitreal injection of the protease inhibitor leupeptin. Although the experimental component of the study was comprehensive, we feel that the interpretation of the results was potentially misleading, particularly because the authors had ignored some fundamental aspects of lipofuscin biochemistry. First, RPE lipofuscin is likely to be heterogeneous, because it will be derived from both autophagy and the degradation of photoreceptor outer segments (POS). This is confirmed by a variety of studies that demonstrate that lipofuscin-like granules naturally accumulate in RPE cells in the absence of POS and that this process can be accelerated by protease inhibitors and certain calcium antagonists. Second, the lipofuscin-like granules generated by protease inhibitors and calcium antagonists in vitro have very different chemical constituents to those of "true" lipofuscin granules isolated from RPE cells. These granules, while exhibiting the broad band fluorescence of lipofuscin, do not contain the classic lipid-soluble fluorophores associated with true lipofuscin. This discrepancy has been highlighted by both ourselves and previously by Katz et al. Third, even when protease inhibitors are used in conjunction with POS, the resultant granules generated in vitro fail to exhibit the classic fluorophores associated with true lipofuscin. Fourth, the recently identified pyridinium bis-retinoid, otherwise termed A2E, has a chemical structure that makes it resistant to lysosomal degradation. This also may be true for other fluorophores within true lipofuscin. Finally, we have loaded cultured RPE cells with true lipofuscin for periods up to 56 days and failed to observe any degradation or change in lipofuscin composition (Boulton M, unpublished results). Although it could be argued that the lysosomal system is less functional in cultured RPE, it is certainly sufficient to digest phagocytosed POS. We feel that the data presented by Katz et al. adds further support to the above raised points. Formation of high levels of large inclusions of lipofuscin-like material was observed in rats shortly after leupeptin administration, but this was not reflected by a corresponding increase in overall cellular fluorescence by 20 weeks. Thus, it is likely that the autofluorescent granules that are transiently, and rapidly, upregulated after leupeptin treatment in the Katz study simply reflect a buildup of phagosomal material (phagosomes are known to be weakly autofluorescent) which is rapidly destroyed once the effect of leupeptin has worn off. The loss of this leupeptin-induced material, together with the observed continuous and equal increase in cellular fluorescence in both the control and treated cell populations after prolonged incubation, is further evidence to the differences in composition between "true" lipofuscin and lipofuscin-like granules.

Finally, a minor but important point: Katz et al. refer to the bimodal accumulation of lipofuscin reported by two studies that were limited by the number of donors in the young and middle-age groups. In vivo fluorescence measurements as undertaken by Delori et al. and Von Ruckman et al. suggest that lipofuscin accumulation is linear throughout life. This is more logical but, sadly, is an observation ignored by many researchers when discussing lipofuscin accumulation.

Mike Boulton
Department of Optometry and Vision Sciences
University of Cardiff
Cardiff, UK
Sallyanne Davies
Steven Ellis
Department of Ophthalmology
Manchester Royal Eye Hospital
Manchester, UK

References


The Authors Respond:

There are a number of points made in the letter from Dr Boulton that we would like to address.

1. Dr Boulton states that "lipofuscin-like granules naturally accumulate in RPE [retinal pigment epithelial] cells in the absence of POS [photoreceptor outer segments]" and concludes that autophagy is a significant source of lipofuscin in the RPE. In support of this contention, he cites several studies that used cultured RPE cells. However, many things that occur in cultured cells do not occur in vivo. With respect to the accumulation of lipofuscin-like autofluorescent inclusions, cell types that do not accumulate lipofuscin in vivo have been reported to accumulate this pigment in culture. Therefore, accumulation of lipofuscin-like inclusions in cultured RPE cells...
is unlikely to be “natural.” We have previously reported that in vivo, very little lipofuscin-like pigment accumulates in the RPE when phagocytosis of outer segments is eliminated early in life.²,³

2. Dr Boulton states that the autofluorescence of lipofuscin-like granules that accumulate in cultured RPE cells is not vitamin A dependent. In contrast to this, we have found that the autofluorescence of the lysosomal inclusions that accumulate in the RPE both during normal aging and as a result of leupeptin treatment are vitamin dependent.⁴,⁵ This difference between cultured RPE and the in vivo situation further supports the conclusion that cultured RPE cells are not a good model for studying “normal” lipofuscin accumulation.

3. Dr Boulton misrepresents the findings reported in one of our earlier publications.⁶ He implies that in this article we reported that the leupeptin-induced inclusions do not contain the classic lipid-soluble fluorophores associated with true lipofuscin. The article he alludes to dealt with lipofuscin-like inclusions that accumulate in response to vitamin E deficiency and not those induced by leupeptin treatment.

4. Dr Boulton cites his own unpublished data indicating that cultured RPE cells loaded with true lipofuscin did not show any degradation or change in lipofuscin composition. This is an interesting finding. We look forward to seeing the data published after it has undergone critical review. However, as stated earlier, in vitro findings may not be applicable to the situation in vivo.

5. In reference to our article, Dr Boulton states that “Formation of high levels of large inclusions of lipofuscin-like material was observed in rats shortly after leupeptin treatment, but this was not reflected by a corresponding increase in cellular fluorescence.” This statement is not accurate, as can be seen from our article.⁷

6. Dr Boulton concludes that the effect of leupeptin “simply reflects a buildup of phagosomal material that is rapidly destroyed once the effect of leupeptin has worn off.” To support this conclusion, he states without documentation that “phagosomes are known to be weakly fluorescent.” Certainly, the POS display no detectable fluorescence in situ at the excitation and emission wavelengths used to demonstrate lipofuscin.⁸,⁹ Therefore, if the phagosomes are indeed weakly fluorescent, it is likely to be because some of their molecular constituents have been incorporated into lipofuscin fluorophores, as we have proposed previously.¹⁰

M. L. Katz
L. M. Rice
C. Gao
Mason Eye Institute
University of Missouri School of Medicine
Columbia, MO

References


TIGR and Stretch in the Trabecular Meshwork

Tumminia and colleagues¹ recently suggested that human trabecular meshwork cells, used to model juxtacanalicular cells, respond to mechanical stretch by an alteration in their cytoskeletal network and signaling cascades. They further postulated that, in the long-term, the pressure-induced signals could cause “altered secretion or degradation through changes in the composition of the extracellular matrix in the extracellular fluid pathway that may be involved in generating outflow resistance.” They state, however, that these mechanisms remain to be defined.

We would suggest that one excellent candidate for their proposed stretch homeostatic mechanism already has been established. The trabecular meshwork-inducible glucocorticoid response protein (TIGR) is produced by trabecular meshwork cells and then is secreted into the outflow pathway. TIGR proteins/glycoproteins could have potentially important interactions with other components of the juxtacanalicular tissue.²,³ This region shows a significant concentration of glycoproteins and glycosaminoglycans and is a likely area for outflow resistance. The shear stress response of TIGR was suggested previously on the basis of structural analysis of the TIGR promoter that revealed four putative sequences that predict its response to shear stress.⁴ In particular, the TIGR protein is predicted to possess characteristics that could influence outflow resistance in response to stress or other stimuli. Characteristics of the TIGR protein include its oligomerization, specific binding to trabecular meshwork cells, and potential interactions with other extracellular matrix molecules in the trabecular meshwork.³ Tumminia et al.¹ also suggest that, in glaucoma, these homeostatic mechanisms may be impaired. Changes in TIGR properties due to structural mutations and/or its increased expression could be one of the mechanisms involving outflow obstruction. Several TIGR structure mutations have been confirmed to associate with glaucoma.¹,⁵ Abnormal stimulation of TIGR gene promoter activity by corticosteroids and oxidative stress were shown to increase TIGR protein expression.⁶ Evidence for the overexpression of TIGR
in glaucoma comes from the observation that approximately 50% of eyes with open-angle glaucoma show notable increases in TIGR staining in the trabecular meshwork compared with age-matched controls. A sequence variation in the TIGR promoter that segregates with affected members in a large pedigree with primary open-angle glaucoma may also be relevant.

In conclusion, the shear stress response of TIGR suggests that the gene could have a role as a fluid flow regulator. The TIGR gene/protein in its cellular and extracellular forms could serve as a homeostatic stretch response mechanism in the normal eye and play a pathological role in the disease state. Ongoing research should further unravel the biology of this interesting gene.

Adam Booth
Thai Nguyen
Jon Polansky
University of California
San Francisco

References


The Authors Respond:

We thank Dr Booth and colleagues for their interest in our article.1 We agree with them that the TIGR/myocilin (TIGR/MYOC) protein is a potentially important new clue for the understanding of pathogenetic mechanisms in glaucoma. In fact, several of us have been involved in elucidating the regulation and action of this protein, and we have reported that there is an increase in the transcription of TIGR/MYOC mRNA in oxidatively stressed trabecular meshwork (TM) cells and in TM cells treated with TGF-β.2,3 In addition, we recently submitted a manuscript showing that TIGR/MYOC mRNA increases in response to applied mechanical stretch.4 We differ somewhat with the statement that the localization of TIGR/MYOC is specific to the TM, because it has been shown that this protein is present in tissues other than the trabecular meshwork.5,6 Nevertheless, TIGR/MYOC may be involved in aqueous outflow and its homeostatic mechanisms. The data obtained from the mechanical stretch experiments4 suggest that the expression of TIGR/MYOC may depend on an inherent balance of the mechanical forces present in the TM. Any alteration in these forces might cause the upregulation and/or downregulation of TIGR/MYOC. Although we too believe that structural mutations of this protein are closely associated with glaucoma, we are not as confident that the overexpression of TIGR/MYOC is the primary mechanism that impedes aqueous outflow. TIGR/MYOC has been found in increasing amounts in the TM of patients with adult-onset primary open-angle glaucoma (POAG) and in patients with pseudoxefolution, a secondary type of open-angle glaucoma.7 Although an increased accumulation of TIGR/MYOC in glaucomatous trabecular meshworks has been observed,7 this increase could represent a secondary phenomenon in which TM cells are attempting to ameliorate an obstruction in outflow. Therefore, the high amounts of TIGR/MYOC present in these eyes may reflect an effect rather than the cause of decreased facility.

Thus, we hypothesize that TIGR/MYOC could play a role as an aqueous humor outflow regulator in outflow pathway cells; however, the regulatory mechanism of TIGR/MYOC has yet to be elucidated.

Santa J. Tumminia
Foundation Fighting Blindness
Hunt Valley, MD
David L. Epstein
Dept. of Ophthalmology
Duke University Eye Center
Durham, NC
Paul Russell
National Eye Institute
National Institutes of Health
Bethesda, MD

References