Author's response to reviews

Title: Epigenetic Histone Methylation Modulates TGFBIp and Extracellular Matrix Gene Expression in Corneal Fibroblasts of Granular corneal dystrophy type 2

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Version: 3
Date: 13 July 2015

Author's response to reviews: see over
Dear Editor-in-Chief,

We are resubmitting our original report entitled “Epigenetic Histone Methylation Modulates TGFBIp and Extracellular Matrix Gene Expression in Corneal Fibroblasts of Granular corneal dystrophy type 2”. All coauthors reviewed the questions, comments, and concerns mentioned by the reviewers and have provided feedback thereto. We acknowledge your efforts to review our manuscript and thank you for the opportunity to resubmit our report for publication. Our point-by-point responses to the reviewers’ questions are listed below. Thank you again for reviewing our manuscript.

Best regards,

Eung Kweon Kim, M.D., Ph.D.

Reviewer: John Halsall
Reviewer's report:
In this paper Maeng and colleagues examine TGFBI signalling in wildtype corneal fibroblasts and in cells carrying mutations in the TGFBI gene which lead to granular corneal dystrophy.

The paper is well presented and clearly and concisely written and the results are of interest. I have made detailed points in my previous reviews of this paper and I thank the authors for their attention to these.

My fundamental concern remains that the interpretation of the results with regard to the functional role of histone epigenetics is incorrect. The authors show that TGFBI gene expression is reduced in mutant cells. They also show that much less TGFBIp is secreted from mutant cells than from wildtype cells but that intracellular TGFBIp is at similar levels in both cell types. Given the reduced secretion of the protein in mutant cells it is logical that less transcription is required to maintain intracellular protein levels and that negative feedback would suppress transcription in mutant cells relative to wildtype. Given this, it is entirely to be expected that levels of H3K4me3, a well-known marker of active transcription, are reduced at the promoter of the TGFBI gene. The authors then show that transcription of TGFBI and associated ECM genes is increased upon treatment with TGF#1 and
H3K4me3 levels again correlate with expression. These effects are reduced but not eliminated in mutant cells. The association of active histone modifications with levels of transcription does not represent proof of a causative link and indeed it seems to me much more likely that the accumulation of TGFBIp is driving the altered transcription pattern in mutant cells. I therefore think that to publish the paper under the current title, suggesting a causative role for histone methylation in TGBI transcription in granular corneal dystrophy, would be misleading. If the paper were to be published without this inference it would make an interesting addition to our understanding of the signalling involved in granular corneal dystrophy.

- Thank you for your comments and suggestions.
- In our previous reports, we have suggested that an insufficient autophagy–lysosome pathway might be responsible for the intracellular accumulation of mutant-TGFBIp during the pathogenesis of GCD2 (Choi SI, Maeng YS et al. Biochem Biophys Res Commun 2014, 450:1505-1511.). We also suggested that newly synthesized TGFBIp was secreted via the endoplasmic reticulum/Golgi-dependent secretory pathway, and this secretion was delayed in the corneal fibroblasts of patients with GCD2 (Choi SI, Maeng YS et al. PLoS One. 2015, 10:e0119561.). Because these two reasons, the level of intracellular TGFBIp was similar in both wild-type and GCD2-homozygous cell though TGFBIp mRNA transcription levels were much lower in GCD2-homozygous cells than that in the wild-type cells. Therefore we suggest that these abnormal conditions caused by mutant-TGFBIp accumulation and delayed secretion within GCD2 cells may induce reduced histone methylation, ultimately decreasing the basal expression of TGFBIp and ECM proteins. Also, this abnormal condition in GCD2 cells may weaken GCD2 response to exogenous stimuli (such as TGFβ1), compared to wild-type cells.

Basically, Granular corneal dystrophy type 2 (GCD2, also called Avellino corneal dystrophy) is an autosomal dominant disorder caused by an arginine-to-histidine substitution at codon 124 (R124H) of the transforming growth factor β-induced gene (TGFBI). Therefore, difference of H3K4 methylation is not causative of GCD2 disease. However, not only mutation of TGFBIp gene, but also regulation of TGFBIp expression is very important to treatment of GCD2. In this reason, new finding of TGFBIp regulation mechanism in granular corneal fibroblast is important to create cornea-protective therapies for granular corneal dystrophy. Although some key transcription factors are known, the epigenetic mechanisms modulating TGFBIp and ECM expression in granular corneal fibroblast is not known. Our findings clearly show that epigenetic histone methylation modulates the expression of TGFBIp and ECM genes in corneal fibroblasts. TGFβ1 strongly increased H3K4me1/3 levels on the TGFBIp and ECM-associated gene promoters and these increases in promoter H3K4me1/3 levels were correlated with the increased expression of the associated gene. In addition, knockdown of MLL1 and
SET7/9 significantly decreased H3K4me3 and H3K4me1 levels on the TGFBIp and ECM gene promoters and subsequently inhibited TGFBIp mRNA levels. These findings provide evidence that active histone H3K4me1/3 modification regulated by both MLL1 and SET7/9 regulate the TGFBIp and ECM expression.

In summary, under extracellular stimuli such as TGFβ1, MLL1 and SET7/9 are activated, increasing their recruitment to SBEs. H3K4me1/3 becomes more methylated, and H3K27me3 becomes demethylated on gene promoters, creating a favorable environment for Smad3 binding, resulting in the increased expression of TGFBIp and ECM-associated genes. Taken together, these our novel epigenetic regulation mechanism of TGFBIp gene and pharmacologic and other therapies that regulate these modifications could be used to cornea protective therapies for granular corneal dystrophy.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests