Background

Age-related macular degeneration (AMD) is one of the largest causes of blindness worldwide. Cigarette smoking is known to be the single largest risk factor for AMD, aside from age. Several of the main genetic risk factors for AMD are polymorphisms occurring in complement genes involved in the alternative, classical and common terminal pathways. To better understand the metabolic impact of smoking on the retina, computational molecular phenotyping (CMP) was used to examine the effects of cigarette smoke on wild type (WT) mouse retinas and retinas of mice in which either the alternative pathway (complement factor B, CfB) or the common terminal pathway (complement component 3, C3) was removed. Specifically, Müller Cells, Retinal Pigment Epithelium (RPE), and photoreceptor Inner and Outer Segments were examined across the aforementioned conditions.

Methods

While smoking is common, obtaining post-mortem tissues from human subjects in a timely fashion is problematic. Therefore, in collaboration with Dr. Baerbel Rohrer’s laboratory at the Medical University of South Carolina (MUSC), we examined the effect of cigarette smoke on the mouse eye. The mouse model is advantageous because the eyes are fully formed in the human eye, with very similar small molecular values in retinal tissues, and serves as a reasonable model for this evaluation. Another advantage of a mouse model is that it allows for alterations in the gene sequence, letting us look at the alterations in the context of the genetic alteration, leading to possible insight on the biological impact these changes have on the retina. To compare the effects of smoke on the retinas, we used an automated smoking machine to generate cigarette smoke (4a). The sections are cut thin enough so that each one of the labels shows signals of antigens from the body. The captured images were registered and clustered to mask out specific cell populations from quantitative statistical analysis. Unsupervised clustering methods, like k-means clustering, allow us to deal with complex data and segment it into manageable classes that can provide statistical measures of separability. We can then focus in on different mathematical cell classes. Here we have considered Photoreceptor Inner Segments (blue), Outer Segments (yellow), RPE (green), and Müller Cells (red). Shown below are some images taken from a wild type mouse that has been exposed to cigarette smoke (4a).

Alterations in retinal small molecule signatures were observed in smoked retinas compared to non-smoked retinas from mice in WT, C3 knockout, and CfB knockout conditions. Histograms were then created from the grey-scale profile for the cell class of interest, which can be used to analyze signal densities of the molecules of interest. These are log plots on the horizontal axis, so small differences are actually substantial. Shown are the concentration profiles for the Inner Segments, Outer Segments, Müller Segments, Müller Cells, and RPE. The plots of particular interest or significance are indicated by a green asterisk.

Conclusions

In response to cigarette smoke exposure, we see substantial metabolic changes in photoreceptors. Glutamate, glutamine, glutathione and taurine are known to be involved in cell stress response & immunoregulation. Glutamate and glutathione increased in photoreceptor cells upon smoke exposure. The enzymatic activities of the complement system, a cascade required for the maintenance of the immune privilege of the eye, appears to exacerbate responses to cigarette smoke in oxidative damage response related pathways. The complete cascade of the complement system via the common terminal pathway (C3 knock out) has a more substantial impact on the inner segments or photoreceptor cell bodies than a partial blockade of the complement system via the alternative pathway (CfB knock out).

The importance of the RPE cannot be understated in the maintenance of photoreceptors while Muller Cells are perhaps best positioned to respond to environmental insults. Glutathione and glutamine are up-regulated in cells undergoing cellular/oxidative stress while arginine up-regulation is a more dramatic effect that the CfB knockout of the feedback loop. There are actually substantial differences in the Müller Cells, RPE and photoreceptor Inner and Outer Segments or photoreceptor cell bodies than a partial blockade of the complement system via the alternative pathway (CfB knock out). These differences are seen in the Müller Cells and RPE of the levels of these small molecules, and of signal variance, seem to indicate that there is some response to the oxidative stress of cigarette smoke that is functional in the C3 knock out that is measurable in the CfB knock out.

Furthuring our understanding of complement-dependent metabolic alterations in the eye might aid in our understanding of AMD pathology and may open new avenues for novel treatment strategies.