cGMP and cGMP-binding proteins as potential therapeutic targets and biomarkers during retinal degeneration

Background
The inherited eye disease retinitis pigmentosa (RP) causes photoreceptor degeneration, which may lead to blindness through a still unknown cell death mechanism(s). Currently, no therapies have yet shown long-term preservation of photoreceptor function and vision in RP patients. The second messenger cGMP is elevated during photoreceptor cell death and interventions with its binding proteins have been shown to reduce photoreceptor degeneration. This suggests cGMP, as well as its binding proteins, as RP disease drivers and their potential as therapeutic targets and/or biomarkers.

Aim and Method
With the emerging need for specific therapeutic targets and biomarkers for retinal degeneration (RD), and thus RP, the general aims of the thesis are to study cGMP-interactors in retinas from RD mouse models (and in mouse brain cortex) as well as cGMP related biomarkers in blood from RP patients. The RD models (i.e. rd1, rd10, and rd2) have different mutations leading to intracellular accumulation of activated cGMP in the degenerating photoreceptors. The outcomes will be compared with control situations (i.e. healthy mouse models or non-RP persons). However, the high complexity of the cellular proteome in the retina challenges the discovery of new targets for drug and biomarker development. Therefore, an optimized affinity chromatography based proteomics approach including cGMP-linked beads will be used to aid the deciphering of retinal cGMP-interactors and their stereo-specific interaction sites, as well as the target specificity of a modified cGMP compound, with already proven photoreceptor protective effects.

Preliminary Results and Significance
An optimized proteomics approach was established and validated and thus was successful in isolating cGMP-interactors from the RD and healthy retinas. Amongst the interactors identified were the previously generally known cGMP-dependent protein kinase (PKG), cAMP-dependent protein kinase (PKA), and various phosphodiesterases (PDEs). However, also proteins that may be new potential cGMP-interactors in the retina were identified, and found to show proximity with cGMP in situ in histological sections. The proteomics approach moreover aided in elucidating cGMP-interactor stereo-specific interaction sites, and together with the information on known and potential new cGMP-interactors this is likely to be valuable when designing new therapeutic strategies against such interactors. Finally, a modified cGMP-analog (with an attached fluorophore), as well as the fluorophore itself, were shown to work as markers for RD in the retina. These reveal new detection methods for photoreceptor cell death, and their potential targets in the retina may help elucidating the photoreceptor degeneration mechanism(s).
Ongoing and Tentative studies

A study which aims to reveal the target specificity of a modified cGMP compound, which previous has been reported to have a photoreceptor protective effects, is ongoing. A follow up-study to elucidate the role of a potential new cGMP-interactor in the retina, namely an exchange factor, is planned. For this study retinal cultures and methods like immunostaining and TUNEL assay will be utilized to try to understand this factor’s role in the degenerating photoreceptors. In addition, a study focusing on peptides (e.g. derived from cGMP-interactors) as potential blood-based biomarkers from RP patients and healthy subjects is being planned.

Publication list

1. Rasmussen M, Welinder C, Schwede F, Ekström P: Analysis of cGMP-beads and isolated cGMP-binding proteins using a chemical proteomics approach in mouse cortex tissue (manuscript in progress)

2. Rasmussen M, Welinder C, Schwede F, Ekström P: A proteomics approach identifies known and potential new cGMP-binding proteins in retinal degeneration and healthy mouse models (submitted)

3. Rasmussen M, Welinder C, Schwede F, Ekström P: FITC and cGMP-F001 as markers for degenerating photoreceptors in the rd1 mouse model (manuscript in progress)