Proteomics

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Goals and Projects:

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a complex disease and the leading cause of blindness in the elderly. Only a fraction of early/mid-stage AMD (also known as “dry” AMD) patients progress to advanced AMD, with neovascular or “wet” AMD being more prevalent than advanced dry AMD (also known as geographic atrophy). Currently clinicians cannot predict which patients will progress to advanced AMD. Objective molecular markers for clinical prognostics could help prevent or slow with severe visual loss. Genomic markers alone are insufficient as many individuals carrying AMD risk genotypes never develop impaired vision. A long-term goal of the laboratory is the development of molecular technology for assessing AMD risk and monitoring AMD therapeutics.

Glaucoma

Glaucoma is a multifactorial optic neuropathy and a leading cause of blindness worldwide. Glaucomatous damage to the visual system can occur at normal and elevated levels of intraocular pressure (IOP). While age and IOP are risk factors for the development and progression of the neuropathy, the identification of additional risk factors for IOP elevation and/or glaucomatous vision loss and their mechanism are a high priority. A long-term goal of the laboratory is to establish a panel of blood-borne biomarkers for glaucomatous damage to the trabecular meshwork (TM) and visual system that will lead to improved clinical assessment of risk for visual loss from primary open angle glaucoma (POAG) and to improved glaucoma patient management.

Research and Innovations

Age-Related Macular Degeneration

Growing evidence supports AMD as an inflammatory disease involving oxidative stress. A host of oxidative protein modifications have been associated with AMD, including modifications derived from lipids such as carboxyethylpyrrole (CEP). Our laboratory participated in the discovery of elevated CEP in AMD ocular tissues and the initial demonstration that CEP stimulates neovascularization, independent of VEGF. Others have shown that oxidative modifications derived from sugars such as carboxymethyllysine (CML) and pentosidine (and known as advanced glycation end products) are elevated in AMD ocular tissues; CML also stimulates neovascularization but through VEGF. Our biomarker analyses have confirmed the AMD biomarker potential of plasma CEP adducts and CEP autoantibodies and demonstrated that CEP and genomic AMD biomarkers used together are more effective in assessing AMD risk than when used alone. Recently we have shown that light-induced CEP adducts in rat retina and plasma is significantly decreased by pretreatment with a serotonin 5-HT_{1A} receptor agonist. These results support CEP biomarkers as possible tools for monitoring the efficacy of select therapeutics. We have also demonstrated the AMD biomarker potential of plasma protein CML and pentosidine. CML plus CEP or CEP plus pentosidine provided significantly improved discrimination accuracy between AMD and controls. Ongoing studies indicate that these markers in combination with genomic AMD markers and CEP significantly improve AMD risk prediction. Using LC MS/MS iTRAQ technology, we have identified proteomic changes with AMD progression in macular Bruch’s membrane/choroid and

established a test set of 99 protein biomarker candidates. We are in the process of verifying AMD biomarker candidates in plasma using targeted quantitative proteomics.

Glaucoma

Previous qualitative proteomic analyses in the laboratory demonstrated that cochlin, a protein associated with deafness, was abnormally expressed in human trabeculectomy tissues from POAG donors, suggesting it possibly could obstruct the aqueous humor (AH) outflow pathway and contribute to elevated IOP. Our previous qualitative proteomic analyses found peptidyl arginine deiminase 2 (PAD2) in human POAG optic nerve. Subsequent analyses demonstrated elevated PAD2 in POAG optic nerve and revealed myelin basic protein as a major deiminated protein, suggesting deimination may contribute to demyelination and visual loss in POAG. We demonstrated the feasibility of global quantitative proteomic analysis of *in vivo* retinal ganglion cells purified from rats with laser induced unilateral experimental glaucoma. Transforming growth factor beta 2 (TGFβ_2) is often elevated in AH and TM of POAG patients. Accordingly, we quantified TGFβ_2-induced proteomic changes *in vitro* in TM cells using LC MS/MS iTRAQ technology and found that TGFβ_2-treatment significantly altered the abundance of TM proteins, including 40 not previously associated with TGFβ_2-signaling in the eye. Glucocorticoids (GCs) are common anti-inflammatory agents that can cause ocular hypertension and secondary glaucoma. Accordingly, we quantified Dexamethasone (Dex)-induced proteomic changes *in vitro* in TM cells and found Dex-treatment also significantly altered the abundance of TM proteins, including 38 not previously associated with GC-signaling in the eye. These results expand the repertoire of proteins known to participate in TGFβ_2-signaling and GC-signaling, demonstrate similarities in proteomic changes induced by steroids and TGFβ_2 and identify glaucoma biomarker candidates. Current global quantitative proteomic studies of AH are directed toward identify biomarker candidates for glaucomatous damage to human TM in patients with ocular hypertension and POAG. In addition we are pursuing global quantitative proteomic analysis of optic nerve head, orbital optic nerve, retina and peripapillary sclera from Rhesus Macaques with laser-induced, unilateral, mild and high IOP early experimental glaucoma. The results from these ongoing global analyses have already identified biomarker candidates for targeted quantitative proteomic analyses of human and monkey glaucomatous and normal plasma. In the long term, this research will verify a subset of biomarker candidates as blood biomarkers for glaucomatous damage to the TM and/or to the visual system.

Lab Staff Members:

- John W. Crabb PhD, *Principal Investigator*
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