Elucidating SNP Mechanisms

Personalized Medicine from Computational Modeling Discovery

Daniel N. Meijles\textsuperscript{1} and Brendan J. Howlin\textsuperscript{2}

Reactive oxygen species (ROS) produced by the NADPH oxidase (Nox) enzymes play a central role in age-related diseases, including Alzheimer’s, type 2 diabetes, hypertension, and heart disease. Despite regulating normal cellular function, ROS, either as a result of cellular stress or from a chronic overload of antioxidant defense mechanisms, negatively impacts cellular health by damaging macromolecules, such as DNA. By extension, ROS cause oxidative damage to tissues and, therefore, remain a dominant proponent for age-related organismal decline. Using a multi-disciplinary approach employing computational protein modeling, molecular and cellular biology, clinical sample phenotyping and characterization, and rational drug design with structure activity relationships, we identify a route of enquiry that uncovers novel mechanisms or drug candidates, which can identify new routes for preventing oxidative stress-linked diseases that acutely correlate with ageing.

Age-Related Diseases and ROS

Advanced human age is an independent risk factor underlying many degenerative diseases, such as Alzheimer’s disease, cancer, arthritis, type-2 diabetes, and cardiovascular diseases including atherosclerosis and hypertension. Moreover, diseases associated with ageing and co-morbidity presentation are acutely correlated. By year 2035, it is predicted that 23% of the UK population (24.3% of EU member states) will be >65yrs and present clinically with one or more age-associated disease, costing health care providers ~£4.4 billion more per year for social care and medical costs [1, 2]. Understanding the mechanisms that lead to tissue degeneration and disease, therefore, are of paramount importance.

A decline in tissue function and a central hallmark of ageing is senescence, a process whereby cells withdraw from mitotic division and lose proliferative responses to growth factors or mitogens. As an irreversible form of cell-cycle...
arrest, cellular senescence is initiated by a variety of stresses including genotoxic and oxidative stress [3].

However, as first observed by Harman and colleagues, oxidative stress has long been considered a key driver of the ageing process [4]. Since their discovery as professional reactive oxygen species (ROS; i.e. superoxide and hydrogen peroxide) producing enzymes, the role of the highly conserved NADPH oxidase (Nox) family is well defined in myriad pathologies where oxidative stress is central in disease aetiology [5]. Only recently, however, has the notion of Nox-derived ROS been proposed as a potential source for age-related oxidative stress [6].

Of the 7 Nox family members, Nox2 remains a prominent source of damaging superoxide in vascular cells and cardiovascular disease. Nox2 generates ROS when the organizing p47\textsuperscript{phox} protein interacts with the Nox2 catalytic core via anchoring with the membrane bound p22\textsuperscript{phox} subunit [7]. Nox2 complex activation, therefore, is highly dependent on post-translational protein modification (e.g. glycosylation and phosphorylation), and occurs in response to multiple stimuli, such as angiotensin-II, tumor necrosis factor-alpha, or elevated glucose levels [8]. Genetic studies using clinical samples identified that the Nox2 complex proteins are highly polymorphic, with many single nucleotide polymorphisms (SNPs) translating to the protein [9]. Recently, natural variation in human ROS levels was hypothesized to result from the effects of SNPs. However, how Nox2-linked SNPs affect oxidative stress-linked diseases with age remains unknown.

**From Protein Modeling to Clinical Characterization**

The p22\textsuperscript{phox} is an essential component of the Nox1-4 isoforms and contains 7 SNPs, of which only 2 translate to the protein. Structurally, using a consensus computational homology modeling approach as no crystal structures exist, it was identified that the p22\textsuperscript{phox} topology contains three N-terminal transmembrane spanning helices and a C-terminal cytoplasmic domain [10], with an extensive
extracellular domain located between helix 2 and 3. As membrane proteins are notoriously challenging to crystalize, a computational modeling approach provided the rational means for understanding structure-function relationship for SNPs of the p22^{phox}, and to generate further hypothesis-driven discovery.

Of the domains in p22^{phox}, the extracellular region positions the site for the clinically relevant C242T SNP, which results in a histidine to tyrosine substitution. Genetically, the C242T SNP is associated with reduced atherosclerotic or hypertensive prevalence in some studies. However, other reports document no effect or that the C242T is linked to vascular disease progression. Given that little consensus existed for the effects of the C242T SNP, a multi-disciplinary approach was used to interrogate the functional consequence of the tyrosine substitution on Nox2 function [8]. Computational protein modeling identified that the tyrosine substitution resulted in a significant structural change in the extracellular loop of p22^{phox}. Using molecular biology approaches and subsequent over-expression of p22^{phox} C242T in human endothelial or p22^{phox}-deficient tumor cells, our work identified an inhibited effect for stimulus-induced Nox2 ROS production. An important regulatory role for the p22^{phox} subunit alongside its ability to anchor p47^{phox} for Nox2 complex assembly is to assist in Nox2 maturation and cytochrome b 558 complex formation. Mechanistically, we identified that the altered structure and inhibited ROS production due to the C242T SNP resulted from reduced maturation and expression of the Nox2 subunit by cell biology and antibody phenotyping techniques. Further, this discovery was functionally interrogated using clinical samples to confirm our in vitro studies and provide clinical characterization. This multi-disciplinary research direction elucidated a novel mechanism for a common SNP mutation and how it is protective against cardiovascular disease via inhibited activation of Nox2.

As the p22^{phox} is essential to agonist-induced ROS production for Nox1 and Nox2 enzymes, both of which are culprits in cardiovascular diseases, we returned to a computational platform to exploit the p22^{phox}-p47^{phox} interface for novel drug discovery [7]. Interestingly, the region for p47^{phox} anchoring by p22^{phox} is located within a region separate to the C242T SNP and has been crystalized. Further work using this multi-disciplinary platform is currently being undertaken to fully characterize potent and entirely novel candidate compounds by structure-activity relationship studies. Our work characterizes for the first time mechanisms that can be exploited by novel drug discovery approaches to inhibit the Nox2 enzyme activity based on inhibited maturation or complex assembly inhibition, thereby providing novel medicaments for attenuating age-related vascular diseases.
Successful treatment of age-related vascular diseases is expected to improve quality of life and reduce the financial burden confronting health services. By elucidating the molecular mechanism of a common Nox2-linked SNP, our research approach enables the design of drugs that will prevent the Nox2 activation process in conditions of stress, including ageing. Importantly, our work has direct implications in support of personalized medicine, which aims to utilize an individual’s genetic makeup for predicting treatment strategies and improve diagnosis. Despite this new branch of medicine being in the discovery phase, multiple examples of targeted strategies exist that are already clinically viable, e.g. trastuzumab (Herceptin) treatment for breast cancer [11]. Given that personalized medicine can be divided into two areas (i.e. influence of genetic variation on drug response or influence of phenotype switches in diseased tissue for variation on drug response) we are able to extend our current knowledge to the effects for inhibiting Nox2 in age-related disease. Firstly, however, viable isoform selective assembly inhibitors (i.e. those currently being studied by our research direction) are essential to global treatment regimens for Nox enzymes in disease aetiology [12]. Next, using the garnered knowledge of the C242T SNP we could tailor efficacy of specific inhibitors to genetic makeup or the need for activity versus expression inhibitors. But, as a consequence of the current research direction in age-related diseases, we must be aware of the interplay between genetics and environment.

Affiliations
1 St. George’s Medical School, Molecular and Clinical Sciences, Research Institute, Department of Vascular Biology, University of London, London, United Kingdom.
2 University of Surrey, Department of Chemistry, Faculty for Engineering and Physical Sciences, Guildford, Surrey, United Kingdom

Contact
Dr. Daniel N. Meijles,
B.Sc (Hons), Ph.D
St. George’s Medical School
Molecular and Clinical Sciences Research Institute
Department of Vascular Biology
University of London
London, United Kingdom
d.meijles@gmail.com
References: