PERSPECTIVE

The use of localized proteomics to identify the drivers of Alzheimer's disease pathogenesis

Alzheimer's disease (AD) is broadly defined by dementia and the presence of specific neuropathological features in the brain (amyloid plaques, neurofibrillary tangles (NFTs) and congophilic amyloid angiopathy). However, the rate of disease progression, type of cognitive impairment, and extent of neuropathology vary widely in patients with AD (Murray et al., 2011). Why this occurs is still unknown, but one could speculate that the cause of this variation is the presence of multiple subtypes of AD, each being driven by different molecular mechanisms.This is consistent with GWAS data suggesting the involvement of multiple different pathways upstream from plaques and NFTs. Better definition of subtypes of AD and improved understanding of the molecular drivers of these subtypes would allow development of more targeted therapeutics, which are urgently needed as current treatments for AD provide only symptomatic relief with no effect on the course of the disease (Wisniewski and Drummond, 2016).

We recently generated proteomic data that supports the concept of multiple subtypes of AD; we found that the protein composition of amyloid plaques was significantly different in patients separated into two subtypes of AD based on the rate of disease progression: those with rapidly progressive AD (rpAD) and those with typical sporadic AD (sAD) (Drummond et al., 2017). Patients with rpAD have a particularly aggressive form of AD where survival is limited to 7–10 months after diagnosis in comparison to a survival time of ~10 years in sAD (Cohen et al., 2015). The molecular mechanisms that underlie this rapid disease progression are currently unknown. Amyloid plaques primarily consist of aggregated beta amyloid (Aβ), and the vast majority of previous research has focused on the causal role of this protein in the development of AD. Importantly, amyloid plaques also contain many other proteins, such as amyloid binding proteins and proteins found in glia and dystrophic neurites that infiltrate plaques, which may also have an important role in the development and propagation of AD neuropathology.

To date, it has been technically challenging to comprehensively analyze protein composition of neuropathological lesions such as amyloid plaques. The majority of studies have relied on targeted protein analysis techniques (primarily immunohistochemistry) to identify proteins found in these lesions. To overcome this challenge we developed our localized proteomics technique, which combines laser capture microdissection (LCM) and label-free quantitative LC-MS to simultaneously quantify expression of hundreds of proteins in an unbiased manner using microscopic amounts of tissue (Drummond et al., 2015). We specifically optimized this technique to allow the use of formalin-fixed paraffin embedded (FFPE) tissue, so that archived human tissue specimens collected at autopsy could be used. This is a particular advantage of our methodology, as the vast majority of human tissue specimens are FFPE blocks, which are currently an underutilized, but exceptionally valuable resource for medical research. Using this technique we found that amyloid plaques contained many proteins besides Aβ, quantifying levels of > 900 proteins in plaques isolated from patients with rpAD and sAD (n = 22/group). 141 proteins had significantly different expression in rpAD and sAD plaques; 85 and 56 proteins had significantly higher and lower expression in rpAD plaques respectively (Drummond et al., 2017). Encouragingly, many of the proteins with significantly altered expression are known to be associated with the development and maintenance of amyloid plaques. For example, there were significantly lower levels of Aβ, gelsolin, and GFAP in rpAD plaques and significantly more α-synuclein, indicating that these proteins have a particularly important role in the rapid development of amyloid plaques in rpAD. This role appeared to be unique to these specific proteins as levels of other important plaque associated proteins (such as tau, ubiquitin and apoE) were similar in rpAD and sAD plaques.

To further understand proteomic differences between rpAD and sAD plaques and to understand how these differences fit into the context of what is already known about AD pathogenesis, we generated multiple new network analysis tools to answer specific questions of interest, as well as using publically available pathway analysis software (Ingenuity Pathway Analysis and STRING). First, we developed a method to determine whether protein differences between rpAD and sAD were predominantly associated with a specific cell type. To do so, we utilized the RNA sequencing dataset recently published by Zhang et al. (2016), which details cell-specific RNA expression in neurons, astrocytes, microglia, oligodendrocytes and endothelial cells isolated directly from healthy adult human temporal cortex. Using this analysis method, we found that rpAD plaques contained significantly higher levels of neuronal proteins and significantly lower levels of astrocyte proteins than sAD plaques. Consequent fluorescent immunohistochemistry showed that the lower levels of astrocyte proteins in rpAD plaques were due to a decreased number of plaque associated astrocytes, but that the higher amount of neuronal proteins was not simply a result of increased numbers of plaque associated dystrophic neurites suggesting that an alternative mechanism was responsible for the increased neuronal proteins in rpAD plaques. Second, we curated a database of all proteins identified in previous proteomic studies to be associated with AD. Proteins were annotated as up- or down-regulated in AD and proteins that are enriched in plaques or NFTs, hence providing a rapid means of comparison with previous proteomic studies. Using this database we showed that proteins with higher expression in rpAD plaques typically have either lower expression in sAD (39% of proteins up-regulated in rpAD plaques are typically down-regulated in sAD) or have no known involvement in sAD (46% up-regulated proteins). This suggests that rpAD is not simply a more extreme version of sAD, but could instead be a separate subtype of AD that is mediated by different pathological mechanisms, consistent with what has been hypothesized in recent studies. Gene ontology analysis found that proteins with altered levels in rpAD plaques were predominantly vesicle proteins and Ingenuity Pathway Analysis suggested that the altered expression of proteins in rpAD was indicative of increased neurotransmission. Together, these results suggest that synaptic vesicle release function may have an important role in plaque formation, and that this process may contribute to the rapid disease progression in rpAD.

A particular advantage of the unbiased nature of localized proteomics is that it allows the detection of numerous novel proteins associated with neuropathological lesions. One such protein detected in our study was secernin-1 (SCRN1). Immunohistochemistry showed that there was low expression of SCRN1 in the brains from age-matched non-demented control subjects, which appeared to be primarily located in the neuronal soma. In contrast, SCRN1 expression was much higher in AD and was found to be specifically accumulated in amyloid plaques, primarily within plaque-associated dystrophic neurites. Expression was similar in sAD and rpAD plaques, hence validating the proteomics results. This is just one example of the discovery of a novel plaque-associated protein using localized proteomics, proving the utility of this technique to further our understanding of the proteins/pathways involved in AD pathogenesis. The large amount of data generated in this study can be used as the basis for future targeted studies to specifically examine the role of each of these proteins in the development of AD.

Our recent study shows that localized proteomics is capable of efficiently identifying many protein differences simultaneously using microscopic amounts of readily available human FFPE tissue. The importance of using human tissue to examine the pathogenesis of AD cannot be stressed enough as AD is a disease that is unique to humans and therefore using human tissue samples to identify drug targets and determine the mechanisms that underlie AD pathogenesis is superior to using tissue from imperfect animal models of AD. As evidence of this, AD clinical trials have had a very high failure rate (~99.6%), in part related to the over reliance on results from preclinical therapeutic studies showing great therapeutic success in

various transgenic AD animal models (Drummond and Wisniewski, 2017). Exploratory studies (such as our proteomic study described here) have the potential to identify disease pathways more directly involved in AD pathogenesis hence, identifying novel drug targets that will be more clinically effective. Our proteomics results correlate well with previous targeted protein studies, validating the use of this technique as a more comprehensive method of analyzing protein differences than traditional targeted techniques such as immunohistochemistry and western blotting; therefore, providing evidence that this technique has great potential for future similar studies to specifically examine protein changes involved in the pathogenesis of a broad spectrum of neurodegenerative diseases.

A small number of previous studies have also successfully used localized proteomics to analyze protein composition of neurodegenerative lesions (*e.g.*, plaques, NFTs, congophilic amyloid angiopathy and Lewy bodies) or specific neuron populations isolated from unfixed, frozen postmortem human tissue (Liao et al., 2004; Wang et al., 2005; Leverenz et al., 2007; Molina et al., 2015; Inoue et al., 2017). The majority of these were proof-of-concept studies showing that localized proteomics was feasible using this type of tissue. Our recent plaque study extends these findings and shows that localized proteomics can be used in larger studies and has the capability of identifying protein differences that define different disease subtypes. The compatibility of our method with FFPE tissue also broadens experimental possibilities going forward because of the increased availability of FFPE postmortem human tissue specimens.

Going forward, the use of localized proteomics has the potential to greatly increase our understanding of the molecular mechanisms that underlie AD heterogeneity and hence allow for subtypes of AD to be better defined based on proteomic differences. It could also help determine whether patients with early onset AD have a similar plaque protein composition to those with sporadic late onset AD, which is a question that currently remains unknown. This will ultimately help with targeted therapeutic development and clinical trial patient selection. We and others have shown that localized proteomics can be used to quantify protein differences in tissue areas even smaller than plaques, such as specific populations of neurons or glia. We are particularly interested in extending our initial findings to determine whether NFT protein composition is also different in different subtypes of AD, whether plaque protein composition changes throughout disease progression, whether plaques and NFTs have the same protein composition in asymptomatic people with extensive neuropathology, and what the proteomic changes are in neurons and glia during the progression of AD. Additionally, localized proteomics could be used to compare the protein composition of neurodegenerative disease lesions in humans and transgenic animal models of disease to identify differences in animal models that could significantly influence translation of results from animal studies to humans. More broadly, this technique can be used to analyze regions or cells of interest isolated from any FFPE tissue, and therefore could be widely used to examine disease pathogenesis across a broad spectrum of diseases.

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Open peer review reports:
Reviewer 1: Hans-Gert Bernstein, Otto-von-Guericke University Magdeburg, Germany.
Comments to the author: This minireview is one of the most exciting manuscripts I had the privilege to review until now. In essence the authors report that plaques obtained from postmortem brains of patients suffering from rpAD (a particularly aggressive form of AD where survival is limited to 7-10 months after AD diagnosis) substantially differ with regard to their chemical composition from plaques from brains of subjects with slowly progressing AD. See details in the additional file.
Reviewer 2: Paulina Carriba, Cardiff University, UK.
Comments to the author: This perspective article shows how different could be the progression of Alzheimer's disease, pointing that such differences could be related to different proteomic alterations. Using a localized proteomics technique that the authors have developed, in which combines laser capture microdissection (LCM) and label-free quantitave LC-MS, the authors are able to analyze the expression of hundreds of proteins using formalin-fixed paraffin embedded (FFPE) tissue. See details in the additional file.
Reviewer 3: Marvin Antonio, Escuela Superrior de Medicina IPN, Mexico.
Comments to the author: The manuscript is interesting and focused in the scope of localized proteomics to identify the drivers of Alzheimer's disease pathogenesis. See details in the additional file.
Additional file: Open peer review report 1–3.

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