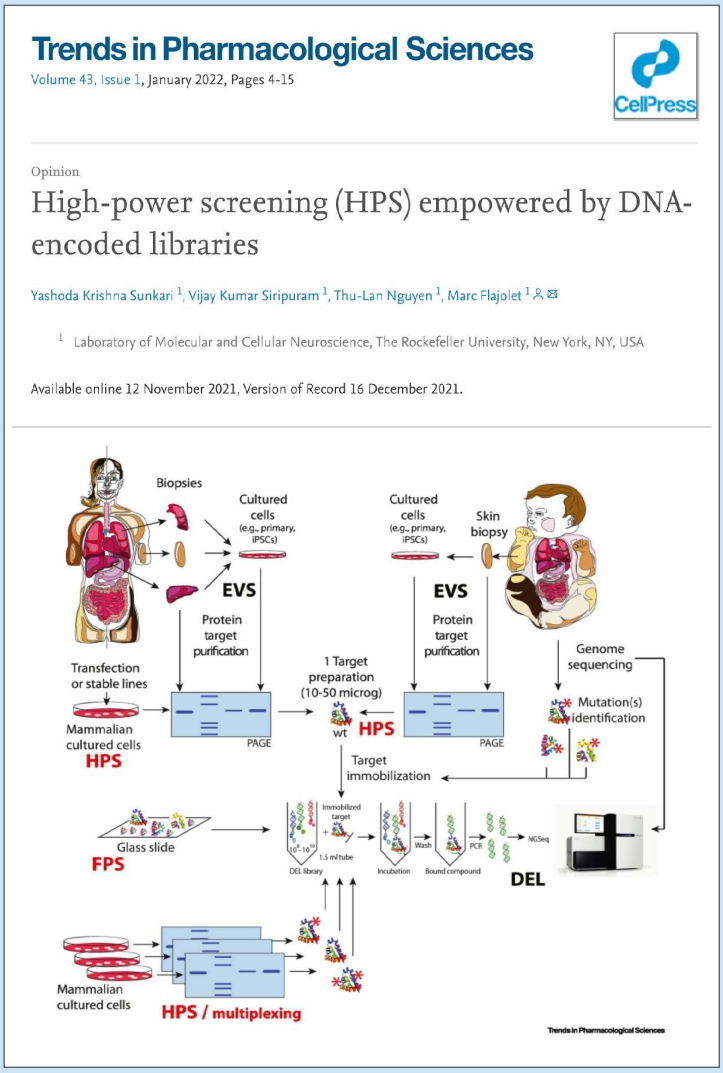
REPORT FROM:

The Fisher Center Lab at The Rockefeller University

Alzheimer's is an extremely complex disease. To address this complexity, Fisher scientists followed their long-standing approach of investigating different complementary aspects of the disease, while focusing more on targeted drug discovery using our own in-house platform.



This year, we made significant progress in five different areas:

- The main part of our project on the selective vulnerability of certain neuron types, which die very early on in the disease was published as we reported, and we will continue this work in collaboration with Dr. Jean-Pierre Roussarie. A follow-up manuscript has been prepared and submitted for publication.
- We made great progress on the development of our drug discovery platform using the DNA-encoded library (DEL) technology. We have published a review/opinion piece in the journal *Trends in Pharmacological Sciences* (see figure above) on the subject.
- We have also published a novel study on the gamma-secretase regulator GSAP in collaboration with the Memorial Sloan Kettering Institute. This work directly follows up one of the projects initiated by us at the Fisher Center; a project very dear to Dr. Paul Greengard.
- We have made significant progress on our drug discovery approach using DEL to identify druglike compounds binding to various types of tau aggregates.
- We completed our work on non-neuronal cells describing how genes linked with Alzheimer's, like PS1, could affect microglia activation.

Analysis of selective cell vulnerability in Alzheimer's Disease

One of our goals was to identify genes/pathways that are implicated in the degeneration of neurons most vulnerable to Alzheimer's. Our strategy is to take advantage of a combination of state-of-the-art tools, and computational and experimental techniques developed by us over many years. We are developing a model system in order to test the role of each of the genes in the degeneration process.

Progress Report:

Over the last reporting period, we pursued our investigation of the molecular mechanisms that lead to the selective degeneration of vulnerable neurons in Alzheimer's disease, as well as our development of tools for the study of these mechanisms, and the identification of therapeutic targets.

Mechanisms of degeneration:

We are about to submit to the journal *"Neuron"* a manuscript about how the protein DEK drives tau pathology in the very vulnerable neurons of the brain. This publication summarizes three years of work funded, in part, by the Stringer Foundation. A very comprehensive study on the links between DEK and tau found that DEK, never before associated with neuronal function, was a crucial regulator of neuron excitability and of tau accumulation. We think that during the course of Alzheimer's, DEK becomes dysregulated and thus contributes to pathological tau accumulation and neurodegeneration. (**Figure 1**)

This discovery of DEK, and of an entire new mechanism involved in Alzheimer's, represents a major proof of concept. We demonstrate that our approach that we have built over the past decade, meticulously investigating vulnerable neurons, and modeling them with computational tools, has the potential to reveal completely novel genes, never before associated with Alzheimer's. We are currently evaluating the possibility and the feasibility to target DEK therapeutically. We are now investigating a few other vulnerability genes that we have identified, all of which might also be potential therapeutic targets. We follow the same experimental framework as with DEK and hope to discover that similar cellular functions are affected by the other vulnerability candidate genes.

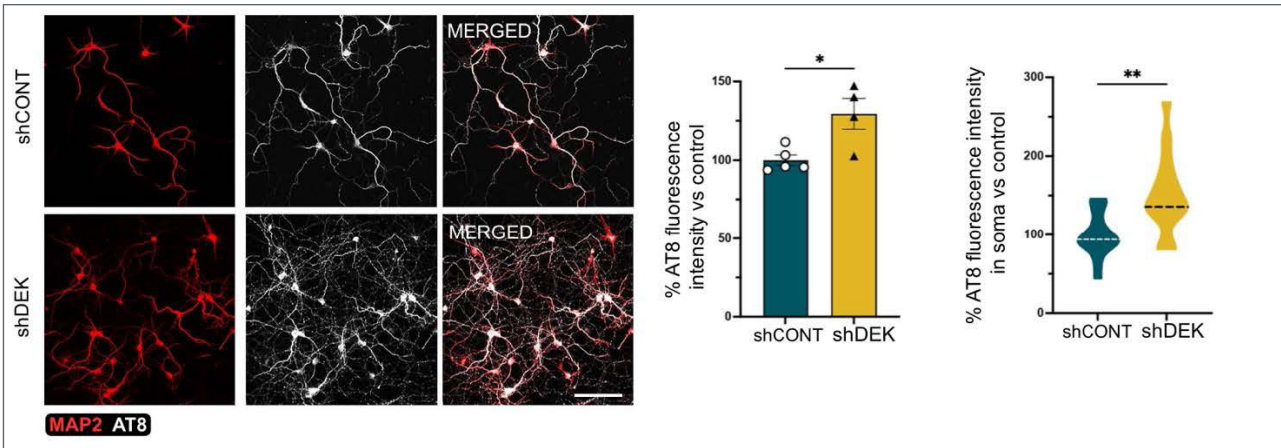


Figure 1: effects of DEK silencing on ECII neurons: the silencing of DEK is increasing the amount of AT8 (phosphorylated tau) in ECII neurons, both the total levels, and the amount in the cell body of the neurons, a sign of pathology.

Description of the early stages of the disease:

In another line of research, we obtained brains from patients that had not been diagnosed for Alzheimer's before their death, and that have no (or very early) amyloid and neurofibrillary tangle pathology (control group). On these brains:

- We are comprehensively studying the modifications of tau present in vulnerable neurons at very early stages of the disease using mass spectrometry.
- We performed single-nucleus RNA-sequencing on these samples and found a few significant differences that are all starting to draw a picture of the early stages of the disease: we find that a specific category of cells is activated before microglia is activated.

We are starting to molecularly dissect the earliest stages of the disease, to identify the earliest events, postulate causation links between dysregulation of different cell types, and to uncover possible intervention points.

Construction, characterization and validation of our in-house drug-screening platform based on the DNA-encoded library (DEL) technology

The development and optimization of methodological alternatives to classical methods such as 'high throughput screening' (HTS) have the potential to significantly accelerate the identification of small molecular weight compounds for a given therapeutic target. This will be extremely valuable, especially for Alzheimer's disease, for which therapeutic interventions remain mostly underdeveloped. The few DNA-encoded libraries that exist today, or first generation DEL, are practically inaccessible and limited to a handful of large pharmaceutical companies.

Progress Report:

We introduced some exciting new features to our state-of-the-art drug screening platform, and are happy to report great progress on that front as well. We have now generated several DEL libraries and have made 125 million drug-like molecules. A patent application has been drafted that includes several of the new features.

Drug screening is often hampered by the absence of a robust biological read-out; however, the DEL technology will circumvent this problem altogether. This means that it is even suitable for targets that are usually extremely difficult, including some Alzheimer's targets, because they do not have an activity (or known activity) that can be measured. One of the novel tools (SELDA) that we have designed and validated is presented in **Figure 2 on the next page** and was used to perform DEL optimization.

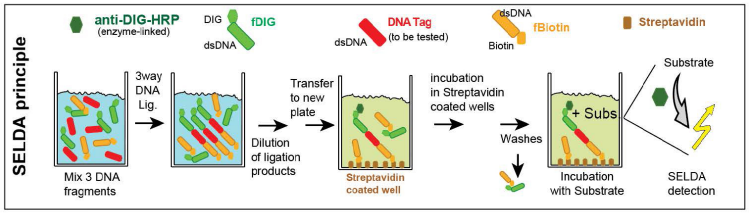
During this reporting period, we have developed and characterized different reagents focused on tau and tau aggregates. We have designed and constructed molecular tools to express the full protein and

have expressed those constructs into human derived cells growing *in vitro* in the lab. A similar approach was used with truncated versions of tau because those are easier to work with and are known to aggregate faster and more easily.

Three series of DEL screens (for a total of 10 screens using 12.5 million compounds) have been performed, and have designed the very first molecules found in our screens that potentially bind to tau.

Visual abstract: SELDA principle

Three types of DNA fragments (fDIG, DNA Tag and fBiotin) are mixed in a tube at equimolar ratio and ligated using T4 DNA ligase. Ligation products are then diluted and incubated in wells of a 96-well streptavidin coated plate. Following washes, ligated products are retained in the wells and fragment ligated on both ends are quantified using horse a radish peroxidase (HRP) based assay.



Visual abstract: SELDA parameters and flexibility

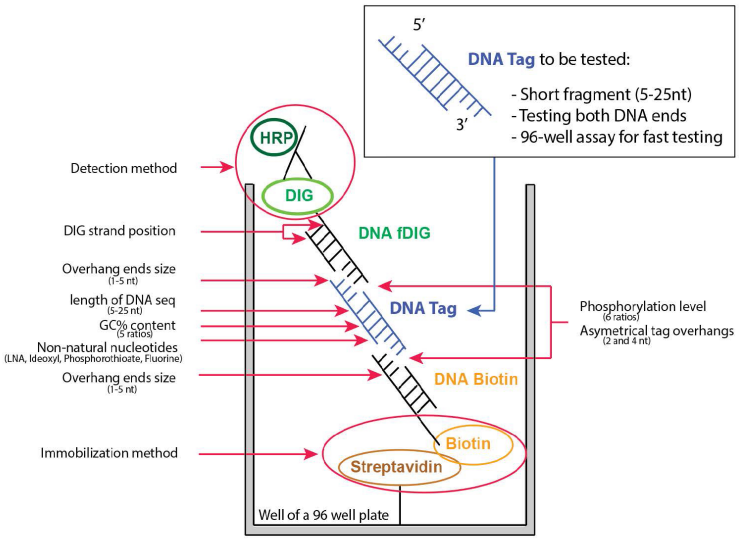


Figure 2 : Schematic representation of a novel tool that we have designed, constructed and validated to improve the quality of our DEL platform.

GSAP regulates lipid homeostasis and mitochondrial function associated with Alzheimer's disease

The existence of amyloid plaques, as the clearest sign of neuropathology in Alzheimer's, has directed recent efforts to the clearance of them. One line of research has emphasized the possible inhibition of gammasecretase. Since the enzyme is responsible for processing APP, inhibition of it was hoped to either slow down or reduce beta-amyloid production. However, feasibility of such an approach has been refuted due to the importance of gamma-secretase in other cellular functions crucial for survival.

Former lab director Paul Greengard thought that finding a specific inhibitor that only targets gamma-secretase's activity would be ideal. Such precise inhibition would preserve the integrity of gamma-secretase in other critical cellular functions, while limiting its negative effects. A few years back, we discovered such a candidate: GSAP (Gamma Secretase Activating Protein). Prior to our discovery, nothing was known about the biological function of this protein and no association with Alzheimer's disease had been made. Our latest study – defining a novel biological function of GSAP – was published in the well-known *Journal of Experimental Medicine*.

Drug discovery approach to identify drug-like compounds binding to various types of tau aggregates

The protein tau, the second most known Alzheimer's culprit, has regained interest after a number

of important clinical trials targeting Abeta plaques have failed. Tau remains at the center of various important biological functions including disease progression, relevance for memory related functions, expression in brain regions involved in learning and memory, and use as a diagnostic tool. For these reasons, a large effort has been put over the years to target tau aggregates, to either attempt removing them or to reduce their production.

Progress Report:

We initiated work to produce tau in cells at the milligram scale, to purify it, to generate aggregates *in-vitro* and to separate them based on their size. The larger aggregates might represent the best target for identifying compounds binding to them, but they might be less important for the disease state. However, compounds binding to larger aggregates might be best suited for diagnostic purposes. The smaller aggregates, also referred to as oligomers, are believed to be the most toxic ones, possibly also largely involved in disease spreading and progression. We made good progress last year, generating tools, testing antibodies, forming and purifying the aggregates, and we have recently initiated several DEL screening campaigns using tau monomers.

We are hoping that DEL molecules that we might identify could be useful for various aspects of tau biology. The most useful application would be to block or reduce tau aggregation as mentioned above. We have two *in-vitro* validation techniques for that goal but we also would like to optimize one method in cells, and perhaps even *in-vivo* in a tau mouse model of Alzheimer's in collaboration with Dr. Roussarie. Our strategy to block tau aggregation should allow us to address the disease at a very early stage and could possibly stop the spreading of tau seeds and the generation of neurofibrillary tangles, blocking the disease progression. Finally, a compound with strong affinity to tau pathological aggregates, if not associated with a relevant biological activity, could still be useful for diagnostic purposes.

Conclusion

In summary, we have made significant progress for each of our projects. **We currently have six manuscripts in preparation.** Three of those manuscripts were submitted and two are under revision and will be resubmitted soon. **The work on vulnerability is progressing extremely well** and we will pursue our effort as outlined previously. A number of improvements for our DEL platform were successful, and new ones are in the works. **We have worked on a large patent application that has now been filed as a provisional patent** application. We will focus now on target preparation and drug screening campaigns with the DEL compounds. We made significant progress on **generating tools and reagents to identify drug-like compounds that could bind to tau**. More importantly, **we are excited to initiate the validation work of the first batch of tau binding compounds identified.**