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Deciphering the molecular logic of ALS using model organisms: "A family affair."

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Abstract

Recent advances in the genetics of ALS have bolstered hope that a molecular logic for the pathogenesis of the disease is fast approaching. An emerging challenge is the dissection of the common and unique molecular pathways altered by ALS gene mutations. Disease modeling in rodents has yielded many important insights, but as the genetic complexity of the disease grows, additional models with improved speed, cost and genetic tractability will be increasingly necessary. Models such as fruitfly, nematode, and zebrafish have been important for diagramming the molecular pathways that underlie many fundamental biological processes, but have been comparatively underutilized in the study of neurodegeneration. Here we highlight the benefits and opportunities for increased diversity in the models used to study ALS.

Introduction

Moving toward a molecular logic for ALS

Even though Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron dysfunction, current developments and discoveries lead the way to a better tomorrow for both patients and clinicians. Initially, the only treatment option was limited to Riluzole, a drug which prolongs life by ~ 3–6 months. However, recently a new drug Redicava received FDA approval [1], and there are more than 3 compounds in Phase III clinical trials (https://clinicaltrials.gov/), the highest number in ALS research history. The compounds that are in clinical trials act upon distinct cellular pathways that are perturbed or dysregulated in ALS patients. For example, Masitinib blocks immune activation in neurons [2], and Redicava is a free-radical scavenger [3]. The challenge in the past was the lack of information on disease causing pathways and compounds to be tested. However the major limitation today is not the absence of data, but understanding the matrix of information generated from many different sources and models [4].

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Previously, ALS was thought to be familial in about 10% of cases and sporadic in the remaining 90% [5]. A number of advancements in genomics including whole-exome sequencing and SNP array have enabled the discovery of numerous ALS genes. Thus far, 36 genes have been identified as “causative” and 107 as “associated” with new ones being added to the list on an almost monthly basis [6]. Especially with the discovery of C9orf 72 GGGGCC repeat expansion in ALS patients world-wide, a high percentage of ALS cases can now be genetically explained [7,8].

To further understand the impact of genetic mutations on motor neuron survival and degeneration, numerous disease models have been generated. The mouse models were the most prominent. Many of the transgenic mouse models that either over-expressed or lacked the mutated form of the human gene were generated with the hopes of recapitulating human condition in a model system. Unfortunately, after 30 consecutive failures in clinical trials, we came to realize that comparing human with mice as a species is not necessarily translational [9,10].

Different model organisms can lead the way

Today there are more than 5 model organisms currently used in ALS research including yeast, nematode, zebrafish, fruitfly, and rodent. Each of these model systems has its unique advantages and strengths. Simple organisms offer enhanced speed, cost, and ease of use and when compared to mice their nervous systems have reduced complexity. Mice have about 100 million neurons, whereas fruitfly flies have 100,000 and nematodes only 302 [11]. There is surprisingly high genomic conservation between species especially in disease genes where mutations are commonly in conserved residues [12]. Therefore, their complexity is indeed an asset to molecular and genetic studies in improving the resolution for analysis of especially complex circuitries.

Many in the field suggested that complex biology cannot be understood by using simple organisms and thus using *S. cerevisiae* or *C. elegans* for a disease such as ALS, would not reflect effective use of time. Contrary to this idea, we have gained an invaluable amount of information even from Baker’s Yeast and this is in part due to many common biology shared between cells and neurons at the cellular, molecular and genetic level [13]. It is also important to remember that many classical studies in fruitfly and nematodes laid the foundation of biology from characterization of nearly all major pathways including Wnt, Notch, and BMP [14,16]. In neuroscience our understanding of synaptic biology depended heavily on the study of *Unc* mutants in *C. elegans* and flightless flies in *Drosophila* [17,18]. So it is indeed possible to obtain valuable information by using simple organisms. Contributions to ALS research by these model organisms have already been very significant with models being generated for SOD1, TDP-43, FUS, Ataxin-2 and more recently in Zebrafish, CCNF and Ubiquilin 4 [11,19 and 20]. These have been very informative and often strikingly recapitulate human disease phenotypes.

Therefore, we, – like many others in the field–, propose to shift our attention from species to cellular biology and focus on the model system that would yield the most reliable and effective solution to the questions we ask. As more ALS genes are discovered how do we continue to diagram and validate the growing ALS gene network? What are the pathways

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that connect ALS genes to each other and which are unique? Understanding with precision the common and disease-gene specific pathways will greatly advance our ability to develop targeted therapies. In order to capture this complexity in the most robust fashion, many have combined model organisms with unbiased genomic tools such as RNA-seq or MS-based proteomics. These have made it possible to elucidate the gene expression changes in motor neurons and other relevant cell types resulting from ALS gene mutation. Such technologies are very powerful in their ability to identify hundreds of differentially expressed genes but often investigators are left with the challenge of generating knowledge from data and revealing the multitude of changes that are most important for the overall disease phenotype.

Every model system has a role to play in the goal of deciphering the ALS genome. One application that would be most advantageous for the use of organisms such as D. melanogaster or C. elegans includes genetic screening to find modifiers of disease phenotypes. Often stocks for the over expression or knockdown via RNAi have been generated by large consortia or individual labs and are available for purchase from centralized repositories [21,22 and 23]. One could use these tools to do unbiased modifier screens. Alternatively, a novel approach would be to limit ones screen the conserved candidate genes generated from transcriptomic or proteomic data. These studies can identify potential pathway members to be used in genetic complementation testing enabling dissection of a molecular pathway. Alternatively, Zebrafish models have made use of high throughput chemical genomic screens to identify small molecules that can perturb biological process [24]. Importantly, these small molecules become tools for the dissection of biological pathways and also starting points for the development of targeted therapies. Additionally, small animal models offer great advantages. First, pathways that are conserved across species are more likely to be important and relevant; second, small animal models give the opportunity to answer biological questions in vivo rather fast and effectively when compared to large animal models or in vitro studies.

The future of ALS research is bright as we now have many disease genes with new ones being added to the list on an almost monthly basis. Deciphering the molecular logic whereby these genes cause ALS should be “a family affair” in which numerous model systems such as the fruitfly, nematode and Zebrafish are equally involved. Currently, >4500 papers have been published on ALS using rodent model, just ~7% of that number have been published using all other small animal models combined [11]. It seems counterintuitive that while these models were paramount for the interrogation of basic biological processes in classical studies, they have been seemingly forgotten in the study of one of the most devastating and challenging diseases, ALS. We foresee a future in which deciphering the molecular logic of ALS using model organisms is indeed “a family affair.”

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Figure:
Using diverse model organisms in a synergistic manner will advance our understanding of the molecular logic of ALS