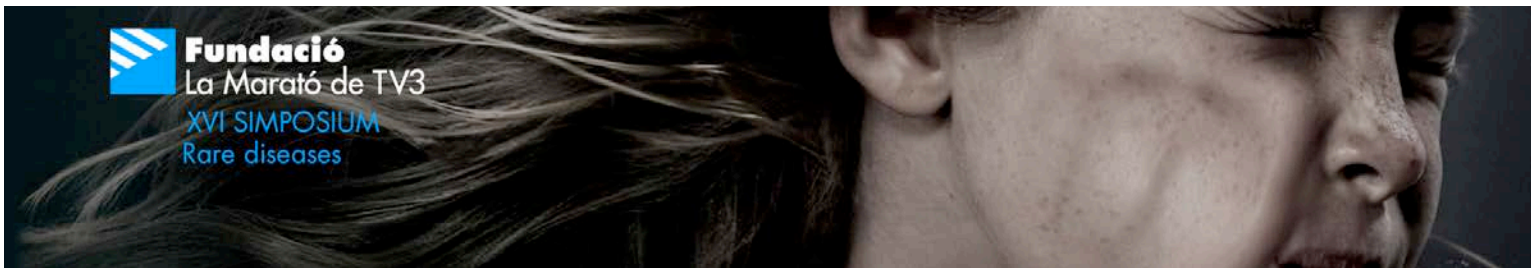

Identification of the androgen receptor aggregates that cause spinal-bulbar muscular atrophy

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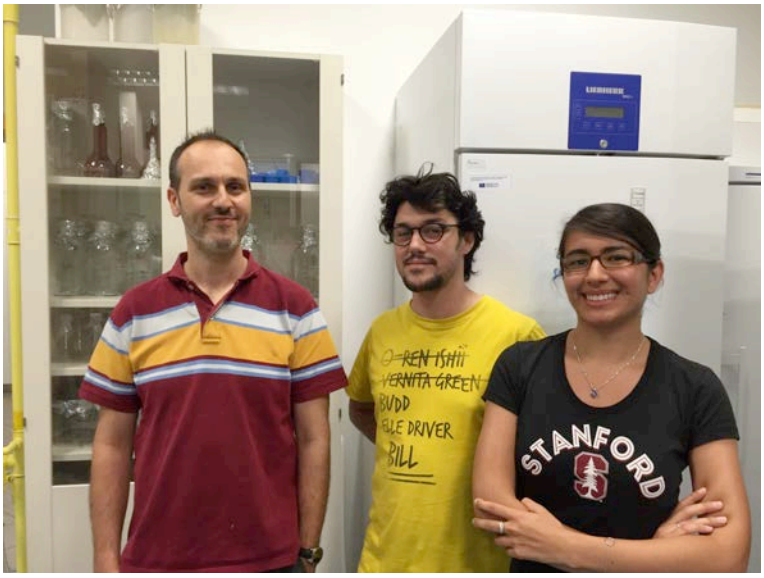

1. Summary of the project

Spinal bulbar muscular atrophy (SBMA) is a hereditary rare neurodegenerative disease that occurs only in men and has no treatment. SBMA was described in 1968 and is caused by the deposition of aggregates of androgen receptor (AR) molecules with an expanded polyglutamine (polyQ) tract in motor neurons.

Formation of such aggregates, of unknown composition, morphology and molecular structure, impairs key cellular functions and leads to disease by motor neuron death in the anterior horn of the spinal cord as well as in brainstem motor nuclei.

SBMA patients present loss of function and gain of function symptoms: the former, such as reduced fertility, are due to an impairing of AR function by an unknown mechanism and the latter, which include weakness of bulbar and extremity muscles, are due instead to the gain of an ill-characterized toxic function by the AR aggregates.

The work that we carried out addressed key aspects of the molecular basis of SBMA that are crucial for the development of therapeutics:

GOAL 1: An in vitro characterization of the structure, dynamics and interactions of the intrinsically disordered N-terminal domain (NTD) of AR that contains the polyQ tract responsible for aggregation and has key functions impaired in SBMA.

GOAL 2: An in vitro characterization of the morphology, molecular structure, stability and neurotoxic properties of aggregates formed by AR and fragments thereof that trigger SBMA. Understanding the origin of neurotoxicity is impossible without an accurate description of the biophysical properties of the aggregates.

GOAL 3: A quantitative determination of the relationship between the formation of aggregates by AR, and fragments thereof, and the degeneration of motor neurons in vivo. To complement the analysis performed in vitro, we quantified the in vivo neurotoxicity associated with changes in AR localization and aggregation.

In summary, by correlating the observations made in vivo with the structural information derived from our biophysical experiments we can determine which types of aggregates, and which features of those aggregates are responsible for motor neuron degeneration in SBMA.

2. Results obtained

Structural properties of the NTD of AR

We have studied the structural properties of the transactivation domain of AR by using nuclear magnetic resonance (NMR), a biophysical technique that is particularly well suited to the characterization of intrinsically disordered (ID) proteins. Our results indicate, in agreement with predictions, that the domain is ID but that that several regions of sequence, known as motifs, are partially folded.

Motifs that are partially folded include the polyQ tract as well as two regions of sequence known as transactivation units 1 and 5, which have long been known to be indispensable for the function of the receptor. These results indicate that the domain is partially folded and that it likely gains structure upon binding to partner proteins, which is a common occurrence in ID proteins.

Equilibrium experiments aimed at studying the oligomerization propensity of the various motifs present in the NTD of AR have indicated that the domain is in equilibrium between a monomeric and an oligomeric form stabilized by

interactions involving well-defined residues. Complementary time-resolved experiments have confirmed that these play a fundamental role in triggering the oligomerization of the domain and the aggregation of AR in SBMA.

We have studied the structural basis of the interaction between the NTD of AR and some of its binding partners such as members of the basal transcriptional machinery and heat shock proteins (HSPs). The former are key for the function of AR and the latter are a key mechanism that protects cells against the detrimental effects of protein aggregation.

The results that we have obtained indicate that the motifs that are partially folded are involved in protein-protein interactions that are key for the function of AR as a transcription factor. Perhaps most importantly they reveal that HSPs bind to the residues that trigger AR oligomerization, providing a simple mechanism for the protective action of these molecular chaperones.

The mechanism of AR oligomerization in vitro and in vivo

Although these were not part of our original plans we carried out experiments in a mouse model of SBMA to determine the nature of the species that aggregate in SBMA. These were in part motivated by a lack of clarity in the literature on whether fragmentation of AR was necessary for aggregate formation in vivo.

In collaboration with Prof. Sobue's group (University of Nagoya) we established a line of transgenic mice expressing human AR with either 24 or 97 glutamine (Q) residues. The mice expressing AR with 24 Qs are indistinguishable from wild type (WT) mice but those expressing AR with 97Qs have severe muscular atrophy and die on average 19 weeks after birth.

We developed a method to extract the AR aggregates from the tissue of the mice expressing AR with 97Qs. This allowed us to determine the tissue where the aggregates form in larger amounts, to analyze their fundamental properties by

transmission electron and atomic force microscopy, and to analyze their composition. We found that they contain proteins other than AR and that the receptor is present as a fragment. Later experiments carried out in cell lines enabled us to establish that the aggregates formed by this AR fragment are taken up by cells, translocated to the nucleus and act toxically.

Having established that AR is fragmented in the tissue where AR fragments are most abundant we carried out experiments in vitro to determine the mechanism by which this fragment oligomerizes and, eventually, aggregates. These experiments showed unequivocally that the mechanism of aggregation consists of two well-defined steps.

In the first step the fragment forms soluble oligomers stabilized by inter-molecular interactions established by specific side chains (see above) and in a second step the soluble oligomers transform into fibrillar species able to recruit additional AR fragments to form toxic aggregates. We found that the rate of formation of the soluble aggregates is relatively independent of the number of Qs present in the tract but that the rate of maturation is critically dependent on it.

Structure/activity relationships in the AR aggregates linked to SBMA

The availability in our lab of a mouse model of SBMA and, most importantly, of a method to extract the AR aggregates from tissues allowed us to establish quite striking relationships between the structure that the aggregates adopt and the consequences that their formation has for the phenotype of the mice.

Our experiments establish that the morphology of the AR aggregates depends on the nature of the tissue where they form and that there is a clear correlation between the number of aggregates formed in a specific tissue of the mice and the severity of the phenotype in the mice.

3. Relevance and possible implications

Our results are relevant for our understanding of the nature of the conformational changes that occur during AR function, for our understanding of the molecular basis of SBMA, and also for the identification of potential therapeutic strategies for combating it. They therefore represent, we think, substantial developments in the field which we plan to publish in the peer-reviewed literature in the near future.

The characterization of the structural properties of the NTD shows that the domain is partially folded and has an intrinsic propensity to oligomerize, which is likely related to its function, and that evolution has favored the development of mechanisms to prevent this propensity to cause detrimental effects. These include the induction of specific secondary structure on the polyQ tract by the regions of sequence flanking the tract as well as the existence of molecular chaperones.

Our observation that the motifs recognized by molecular chaperones are the same as those suggested by our experiments as key for AR oligomerization strongly suggests that they represent a therapeutic target for combating SBMA. With this mind we are currently setting up a biophysical assay for the identification of small or biological molecules with the ability to identify these motifs and interfere with oligomerization.

In addition to putting forward a potential therapeutic strategy these results strengthen the hypothesis that modulators of the activity of molecular chaperones have the potential to be very beneficial for SBMA patients because they indirectly prevent the establishment of the inter-molecular interactions that lead to the formation of toxic AR aggregates.

The observation that the accumulation of toxic AR aggregates with well-defined composition and structural properties occurs in specific tissues and that their mass correlates with the phenotype observed in the mouse model strongly suggests that they are responsible for the symptoms observed in patients. This has important implications because it defines this specific tissue as the one that needs to be targeted in the future by therapeutics.

In summary, we have obtained results that provide the identity of both the tissue where the toxic effects are exerted in SBMA and of the AR motif involved in the inter-molecular interactions that trigger the oligomerization process associated with this disease. Encouraged by these results we are now pursuing studies to further validate them with the aim, in the future, to contribute to drug development.

4. Literature generated

We are at present preparing several papers for publication in which we will present the details of the findings that are outlined in this document.