THE HIGHLY HETEROGENEOUS SPINOCEREBELLAR ATAXIAS: FROM GENES TO TARGETS FOR THERAPEUTIC INTERVENTION.

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Spinocerebellar ataxias (SCAs), also known as autosomal dominant cerebellar ataxias (ADCAs), comprise a highly heterogeneous group of inherited neurodegenerative disorders consisting principally of progressive motor difficulties such as ataxia and loss of balance and motor coordination that result from progressive degeneration of Purkinje cells in the cerebellum and neurons of the brain stem. The age of onset of the clinical symptoms is usually within the 3rd or 4th decade of life and unfortunately, there is no effective therapy for any SCA yet.

Because of the high heterogeneity and overlapping clinical symptoms, the classification of hereditary ataxias has not been straightforward. Traditionally, the different ADCAs have been included in three different groups based on the associated signs. ADCA I presenting optic atrophy, ophthalmoplegia, which is paralysis of the muscles of the eye, pyramidal and extrapyramidal signs, cognitive impairment or peripheral neuropathy; ADCA II, characterised by degeneration in the retina; and ADCA III, with absence of associated signs. The identification of the genes responsible for the dominantly inherited cerebellar degeneration using molecular studies has provided a new classification based on genotype and pathogenic mechanisms (Table 1). In a few SCA subtypes the molecular defect consists of a polyglutamine expansion in the disease protein that is produced as a result of the expansion of an unstable CAG repeat located within the coding region of the disease gene. This is the case of the SCAs 1, 2, 3/Machado-Joseph disease, 6, 7, 17 and DRPLA (SCAs of type 1). In a second group of SCAs, including SCAs 8, 10 and 12 (SCAs of type 2), the mutation consists of a repeat expansion located outside of the coding region of the disease gene. In a third group including two novel SCAs recently described (SCAs of type 3), the genetic defect does not consist of a genetic expansion of an unstable repeat in the DNA. It appears that alterations in amino acid composition dysregulating protein function cause cerebellar ataxia in these types of SCAs. In the rest of the SCAs (SCAs 4, 5, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22), the gene and, therefore, the mutation remain to be identified and characterised. Finding the genes associated with the SCAs and, more importantly, understanding the molecular mechanisms by which the mutation induces the disease symptoms should allow us to discover potential targets for therapeutic intervention.

During the last decade much effort has been dedicated to the investigation of the pathogenic mechanisms underlying the different SCA subtypes. Most of the work has been predominantly done in SCA1, SCA3, SCA7 and DRPLA, which are caused by polyglutamine expansions in the disease proteins. Studies in cell culture, in vertebrate and mouse models have revealed that the disease in these types of SCAs most likely results from the toxic effects caused by the mutation in the disease proteins. These studies have also provided evidence supporting that protein misfolding, subcellular mislocalisation, alterations in protein solubility, aggregation and clearance are fundamental defects conferred by the polyglutamine expansion in mutant ataxin. Importantly, the identification of numerous ataxin-interacting proteins allows a better understanding of the cellular pathways that are vulnerable to the toxic insults exerted by the expanded polyglutamine. From these studies we know now that perturbation of the pathways by which ataxins exert their function appears to be the primary causative cellular defect that triggers neuronal dysfunction in SCAs. In addition, we also obtain evidence indicating that common cellular pathways are responsible for different types of SCAs. The challenge now is to determine how these responses account for the clinical manifestation of the disease, and ultimately, how this knowledge can facilitate the development of therapeutic drugs.

We know now that several cellular pathways are susceptible to be targeted for therapeutic treatment. Since the polyglutamine expansion confers novel conformational features to the disease protein, it is not unexpected that the chaperone system is involved in the cellular response to polyglutamine pathology in those SCAs caused by expanded polyglutamines. The chaperones comprise a group of conserved enzymes whose roles include facilitation of protein folding *in vivo*. In particular, chaperones HSP40 and HSP70 [HSP: heat shock proteins] have been found in aggregates with mutant ataxin-1 or ataxin-3 in mice and flies. Because HSP70 overexpression diminishes cellular toxicity in several animal models, agents that enhance HSP expression are promising potential candidates as therapeutic agents. Indeed, several chemical chaperones such as DMSO, glycerol and trimethylamine *N*-oxide (TMAO) are known to prevent aggregation and cell toxicity induced by a truncated mutated form of ataxin-3. More recently, attention has focused on screens for drugs that prevent aggregation of proteins with expanded polyglutamines. Certain peptides and chemicals such as Congo red, thioflavine S, chrysamine G and Direct fast yellow are able to inhibit aggregation of proteins containing expanded polyglutamine tracts. All these compounds have affinity for structures of the type amyloid ß-sheet, a particular type of protein structure detected in brains of patients with Alzheimer's disease, and, therefore, could be tested to prevent aggregate formation in those types of SCAs caused by polyglutamine expansions.

Anomalies in protein clearance by the ubiq-

uitin/proteasome pathway (UPP) play a role in the cellular response to the expanded polyglutamine in SCAs. Ubiquitin and proteasome components are found associated with aggregates containing mutant ataxins or fragments of them, in both disease models and patient tissues, suggesting that while neurons attempt the degradation of mutant proteins by the UPP, the polyglutamine expansion likely confers resistance to proteasomal degradation. Therefore, chemicals that regulate the UPP pathway, for instance by enhancing proteasomal degradation of mutant proteins, might be potentially effective in diminishing aggregation and, consequently, preventing cellular toxicity and could be potentially used in therapeutic strategies. By a different mechanism, cleavage of some ataxins, including ataxin-3 in SCA3, by caspases, a group of potent proteases, can activate specific pathways of cell injury in neurons. Therefore, inhibition with chemicals such as zVAD-fmk, CrmA, FADD DN and minocycline could be targeted to prevent disease progression.

Recent evidence has shown that transcriptional dysregulation is a relevant event in the pathogenesis of polyglutamine-induced neurodegeneration in SCAs and an early target of polyglutamine toxicity. In support of this view, polyglutamine expansions in the general transcription initiation factor TATA-binding protein (TBP) are responsible for SCA17. In addition, several nuclear factors that are implicated in gene transcription including the leucine-rich acidic nuclear protein (LANP), the *Drosophila* eyes-absent protein, $TAF_{II}130$, mSin3A, the cone-rod homeobox protein (CRX), CREB-binding protein (CBP), p300 and PQBP-1 have been identified based on their interactions with some ataxins. The interaction with the expanded polyglutamine seems to antagonize or alter the function of such nuclear factors thus becoming detrimental on transcription in neurons. With such direct and specific interference of transcription by expanded polyglutamine in ataxins and the indirect consequences on alterations in gene expression, it is feasible to develop drugs that alleviate the negative effects of glutamine expansions on specific pathways of gene expression. Thus, genetic screens for enhancers and suppressors of toxicity in SCA1 flies have identified several transcriptional cofactors as modifiers of polyglutamine toxicity, including dCtBP, dSir2, Rpd3, Sin3A and ter94. Recent findings showing that alterations of gene transcription are caused by undesirable effects of the polyglutamine expansion have encouraged the use of the chemical suberoylanilide hydroxamic acid (SAHA) in pre-clinical trials. These studies have shown that chemical incorporation of acetyl groups into histones, a group of proteins that bind DNA to form the chromatin of the cell, by a particular group of enzymes known as histone acetylases, reduces toxicity and ameliorates the motor deficits caused by expanded polyglutamine in flies and mice. While these encouraging findings suggest that restoration of the levels of acetylation in histones with drugs that inhibit histone deacetylation (HDAC) could prove effective in some forms of Spinocerebellar ataxia, it appears that these drugs interfere with diverse actions on cell survival, proliferation, differentiation and apoptosis by affecting gene expression. Therefore, to prevent non-desirable effects on gene transcription by HDAC inhibitors, specific and effective agents inhibiting deacetylation on selective chromatin (see above) regions should be developed before proceeding to clinical trials.

The involvement of calcium-dependent pathways in the pathogenic mechanisms of some SCAs is supported by several observations. In SCA1, some neuronal genes that are involved in the regulation of the levels of calcium are dysregulated in mice and brain tissues from affected patients. In SCA6, mutations in the alpha 1A-voltage-dependent calcium channel subunit are found associated with the disease. Importantly, the discovery that ataxia in tottering and leaner mice is caused by mutations in the *SCA6* gene allowed to compare the electrophysiological properties of the wild-type and mutant channels. These experiments demonstrated that the mutations are responsible for the neuropathic phenotypes of reduction in current density and the alterations in channel function, which ultimately lead to neuronal death and cerebellar atrophy. Therefore, preventing the perturbation of the intracellular calcium signalling pathways is a possible target of therapeutic intervention.

So far, I have discussed the molecular mechanisms involved in those SCAs where the polyglutamine expansion is the primary causative effect of neurodegeneration since they have been object of intensive investigation. However, in most of the SCAs, the pathogenic mechanisms remain unknown because either the disease gene remains to be identified or the mutation does not consist of an expanded polyglutamine in the disease protein. In SCA8, the *SCA8* gene itself is not translated into protein, but seems to overlap with the transcription and translation start sites and the first splice junction of KLHL1, a gene that encodes a protein with structural similarities to a family of factors involved in the organisation of the cytoskeleton, which is the network of protein filaments and tubules in the cytoplasm in charge of providing shape and coherence to the cells. Although the mode of pathogenesis in SCA8 remains unclear, it has been hypothesized that SCA8 normally regulates expression of KLHL1 at the RNA level. Therefore, abolishment of KLHL1 expression by the repeat expansion would disorganise the cellular skeleton in affected brain cells. If this is the case, modulation of KLHL1 activity could be of potential therapeutic benefit.

An intronic expansion of a highly unstable ATTCT pentanucleotide repeat within E46L, a novel gene of unknown function, is responsible for SCA10, an infrequent SCA form found in some families of Mexican-Hispanic origin. Although the pathogenic mechanisms in SCA10 are unknown, it seems plausible that the expanded repeat could affect the expression of the SCA10

Gene	ADCA	Gene/Locus	Protein	Mutation type
SCA1	I	6p22.3	Ataxin-1	Polyglutamine
SCA2	I	12q23-24.1	Ataxin-2	Polyglutamine
SCA3/MJD	I	14q32.1	Ataxin-3	Polyglutamine
SCA4	I/III	16q22.1	?	Unknown
SCA5	I/III	11p12-q12	?	Unknown
SCA6	III	19p13.2	CACNAIA	Polyglutamine
SCA7	II	3p12-13	Ataxin-7	Polyglutamine
SCA8	I	13q21	KLHL1	Untranslated CTG
SCA9	?	?	?	Unknown
SCA10	*	22q13-qter	E46	Intronic ATTCT
SCA11	III	15q14-21.3	?	Unknown
SCA12	I	5q32	PPP2R2B	5' UTR CAG
SCA13	*	19q13.3-13.4	?	Unknown
SCA14	III	19q13.4-qter	?	Unknown
SCA15	III	3p24.2-3pter	?	Unknown
SCA16	III	8q22.1-24.1	?	Unknown
SCA17	*	6q27	TBP	Polyglutamine
SCA18	I/III	?	?	Unknown
SCA19	I	1p21-q21	?	Unknown
SCA20	I	?	?	Unknown
SCA21	I	7p21.3-p15.1	?	Unknown
SCA22	III	1p21-q23	?	Unknown
SCA**	I	13q33.1	FGF14	Missense mutations
SCA**	I	19q13.4-qter	PKC gamma	Missense mutations
DRPLA	I	12p13.31	Atrophin	Polyglutamine

*SCA10 with epilepsy and seizures, SCA13 with mental retardation and SCA17 with dementia should probably be included in a novel category of ADCAs.
**SCA subtype not assigned yet.

Table 1. Classification of Spinocerebellar ataxias

gene in a fashion similar to the effect of the repeat in Friedreich's ataxia. Elucidation of the function of E46L should provide insights into the pathogenic mechanisms of the repeat in SCA10.

A CAG repeat located in the regulatory region of the *PPP2R2B* gene which encodes a regulatory subunit of protein phosphatase 2A (PP2A) has been associated with SCA12. Protein phosphatase 2 has been implicated in the regulation of many cellular processes including cell growth and differentiation, DNA replication, cellular morphogenesis and apoptosis. It seems plausible that alterations in the expression of PPP2R2B by the SCA12 mutation could in turn shift the substrate preference for PP2A, with potentially lethal consequences. Of particular interest are the findings that PP2A is implicated in Alzheimer's disease and might play a pathogenic role in SCA1, providing evidence that common cellular pathways and similar pathogenic mechanisms might be underlying degeneration in several SCA subforms. A better understanding of the cellular pathways by which PP2A exerts its function should lead to find therapeutic targets in some forms of cerebellar ataxia.

Recent elucidation of the molecular defects in two novel forms of SCA provide evidence of alternative mechanisms of neuronal dysfunction and cerebellar degeneration. Alterations in protein stability of fibroblast growth factor 14 (FGF14) underlie neurodegeneration of the cerebellum and basal ganglia involved in a novel non-episodic form of SCA. In another type of SCA, changes in the amino acid composition of protein kinase C gamma (PKC gamma), an enzyme that regulates cell death through several mechanisms, abrogating either the zinc-binding or phorbol ester-binding capabilities of the protein appear to be responsible for cerebellar ataxia. Interestingly, immunohistochemical studies on cerebellar tissue from an affected member demonstrated reduced staining for both PKC and ataxin-1 in cerebellar Purkinje cells, which are the cells degenerating in SCAs responsible for ataxia and motor deficits. These results suggest that there may be a common pathway for PKC gamma- and polyglutamine-related neurodegeneration further supporting that common pathways are responsible for neurodegeneration in different SCA subtypes. These findings are very relevant for understanding the cellular mechanisms underlying cerebellar ataxia.

In 10 years, since the first gene associated with Spinocerebellar ataxia was identified, our efforts dedicated to discover new SCA genes, characterise new mutations and investigate the molecular basis of the disease have provided enormous advances in our understanding of the complex molecular events that lead to neuronal degeneration in Spinocerebellar ataxias. However, still much work needs to be done. We, as researchers, should now focus our goals to determine an accurate prevalence of SCAs in Europe in order to improve the studies of those SCA forms that are less frequent and, therefore, less characterised. We should also find the genes that remain to be identified and generate more SCA animal models that can be used for the characterisation of novel ataxins and for assessing

the effects of the mutations on their modes of action. But most importantly, we should intensify our efforts to design effective therapeutic strategies. Studies aimed to understand the effects of the mutations on the function of ataxins and to decipher the corresponding cellular pathways implicated will facilitate the identification of molecular targets for therapeutic intervention. Translation of the results of these studies into pre-clinical and clinical trials should bring such an important goal of finding effective treatment for SCA patients to fruition.