FET proteins in frontotemporal dementia and amyotrophic lateral sclerosis

Ian R. A. Mackenzie^{a,*}, Manuela Neumann^b

^aDepartment of Pathology
Vancouver General Hospital
855 West 12th Avenue
Vancouver, BC
V5Z 1M9 Canada
Tel: 1-604-875-4480
Fax: 1-604-875-5707
ian.mackenzie@vch.ca

^bInstitute of Neuropathology
University Hospital of Zürich
Schmelzbergstr. 12
CH-8091 Zurich
Switzerland

* corresponding author

Abstract

Mutations in the fused in sarcoma gene (*FUS*) cause amyotrophic lateral sclerosis (ALS) with TDP-43-negative, FUS-positive pathology. FUS is also the pathological protein in most tau/TDP-43-negative subtypes of frontotemporal lobar degeneration (FTLD-FUS). FUS, together with Ewing's sarcoma protein (EWS) and TATA-binding protein associated factor 15 (TAF15), make up the FET family of DNA/RNA binding proteins that share functional homology and have the potential to interact. We recently investigated the role of the other FET proteins in the clinicopathological spectrum of FUS-opathies. In all FTLD-FUS subtypes, FUS-positive pathology was also labeled for TAF15 and EWS and cells with inclusions showed a reduction in the normal nuclear staining of all FET proteins. In contrast, in cases of ALS-*FUS*, TAF15 and EWS remained localized to the nucleus and did not label FUS-positive inclusions. Cell culture models replicated the human diseases. These findings indicate that ALS-*FUS* and FTLD-FUS have different pathomechanisms and add TAF15 and EWS to the growing list of RNA-binding proteins involved in neurodegeneration.

Key words: frontotemporal dementia, amyotrophic lateral sclerosis, FET, FUS, EWS, TAF15.

1. Introduction

Frontotemporal dementia (FTD) is a clinical syndrome in which the associated neuropathology (referred to as frontotemporal lobar degeneration, FTLD) is heterogeneous. Although most cases of FTLD are characterized by the abnormal intracellular accumulation of either tau or TDP-43 protein (FTLD-tau, FTLD-TDP respectively), there remains ~10% of cases, composed of a heterogeneous collection of poorly characterized conditions, for which the molecular basis was previously unknown (Neumann et al., 2009c). Following the discovery of mutations in the fused in sarcoma gene (*FUS*) as the cause of amyotrophic lateral sclerosis (ALS) type 6 (Kwiatkowski et al., 2009; Vance et al., 2009), we investigated the role of FUS and other FET proteins in the pathogenesis of the tau/TDP-negative FTLD subtypes.

2. FUS in FTD and ALS

2.1. FUS is the pathological protein in most tau/TDP-43-negative FTLD

In a series of studies, using immunohistochemistry (IHC) and immunofluorescence (IF) techniques, we determined that the conditions known as atypical FTLD with ubiquitinated inclusions (aFTLD-U), basophilic inclusion body disease (BIBD) and neuronal intermediate filament inclusion disease (NIFID) are all characterized by FUS-immunoreactive (FUS-ir) neuronal and glial inclusions (Munoz et al., 2009; Neumann et al., 2009a, 2009b). Although these conditions show significant clinical and pathological overlap, we identified sufficient differences in the patterns of FUS-ir pathology to recommend that they should continue to be considered closely related but distinct entities (Mackenzie et al., 2011b). Immunoblot analysis demonstrated a relative shift of

FUS to the insoluble protein fraction, but no abnormal M_r species (Neumann et al., 2009a). Molecular genetic analysis failed to identify any *FUS* gene abnormality in our cases (Neumann et al., 2009a, 2009b). Subsequent studies of large numbers of cases from many international centers, confirmed that most cases of tau/TDP-43-negative FTLD have FUS-positive pathology (Urwin et al., 2010). As a result of these findings, the neuropathological classification system for FTLD was revised in 2010, with aFTLD-U, NIFID and BIBD being subsumed under the new molecular category of FTLD-FUS (Mackenzie et al. 2010).

2.2. Distinct patterns of FUS pathology in ALS-FUS

In a separate study, we reviewed a series of six cases of ALS with *FUS* mutations (ALS-*FUS*) and identified two distinct patterns of pathology that correlated with specific *FUS* mutations and the clinical course (Mackenzie et al., 2011a). Cases with juvenile onset and rapid progression, that included two with the P525L mutation, were characterized by frequent basophilic inclusions (BI), round FUS-ir neuronal cytoplasmic inclusions (NCI) and rare glial cytoplasmic inclusions (GCI). In contrast, cases that onset in mid-adult life with a longer duration, including two with the R521C mutation, had very few BI, a predominance of filamentous FUS-ir NCI and abundant FUS-ir GCI. Neither of the patterns of FUS-ir pathology found in ALS-*FUS* matched those characteristic of the various FTLD-FUS subtypes, including BIBD (Mackenzie et al., 2011b).

These different patterns of pathology, particularly the variability in the frequency of BI, may be explained by recent *in vitro* studies that have examined the functional consequences of different *FUS* mutations (Bosco et al., 2010; Dormann et al., 2010).

Most pathogenic *FUS* mutations disrupt the nuclear localization signal and result in relative redistribution of FUS to the cytoplasm where it is recruited into stress granules, cytosolic structures that temporarily store mRNA during cellular stress. With persistent high cytosolic levels of FUS and prolonged stress, these stress granules grow, coalesce and eventually form large cytoplasmic inclusions. BI contain both mRNA and stress granule markers and may represent the endstage of this process. The fact that different *FUS* mutations affect nuclear import to differing degrees (i.e. the P525L mutation results in severe disruption while R521C causes relatively little impairment), likely explains the variations in the associated clinical course and the extent of BI and FUS-ir pathology in the cases we examined.

3. Other FET proteins in FUS-opathies

FUS belongs to a group of DNA/RNA binding proteins, known as the FET protein family, that also includes Ewing's sarcoma protein (EWS) and TATA-binding protein associated factor 15 (TAF15) (Law et al., 2006). All of the FET proteins were initially discovered as components of fusion oncogenes that cause specific types of human cancer. They are all involved in various aspects of RNA metabolism and are predominantly nuclear in localization, but shuttle between nucleus and cytoplasm. Protein-interaction studies suggest that the FET proteins are able to interact with each other and form protein complexes. This suggests that alterations of TAF15 and EWS might also contribute to the pathogenesis of FUS-opathies. To investigate this possibility, we performed IHC, IF, biochemical and molecular genetic analysis of TAF15 and EWS in a series of FTLD-FUS and ALS-FUS cases (Neumann et al., 2011).

3.1. All FET proteins co-localize in pathological inclusions in FTLD-FUS

We found that all FTLD-FUS subtypes showed strong TAF15 immunoreactivity of all types of neuronal and glial cytoplasmic and intranuclear inclusions (Fig. 1a, b). Double label IF confirmed near complete co-localization of TAF15 and FUS. In addition, cells with inclusions showed a consistent loss of the normal physiological nuclear TAF15 staining. Similar findings were obtained with antibodies against EWS, although there was more variability in the sensitivity of the different antibodies and the intensity of staining among different cases. In all FTLD-FUS subtypes, at least a proportion of each of the different types of cellular inclusions was immunoreactive for EWS (Fig. 1c).

Double label IF showed more consistent co-localization of EWS and FUS in cases of NIFID and BIBD; whereas, in aFTLD-U, only a portion of the FUS-ir inclusions were also EWS positive. The staining variability made it more difficult to evaluate the intensity of nuclear EWS staining; however, there appeared to be some reduction in cells with inclusion bodies. Immunoblot analysis showed a clear shift of FUS and TAF15 to the insoluble fraction with a similar trend for EWS. Genetic analysis of eight cases failed to identify any coding variants in any of the FET genes.

3.2. Selective accumulation of FUS in pathological inclusions in ALS-FUS

Strikingly different results were obtained when we investigated six cases of ALS-FUS, which included four different *FUS* mutations (Neumann et al., 2011). Although all cases had numerous cellular inclusions that labeled intensely for FUS, there was no abnormal immunostaining demonstrated with any of the TAF15 or EWS antibodies. None of the

FUS-ir inclusions labeled for either TAF15 or EWS and all cells showed retention of the normal nuclear TAF15 and EWS staining.

3.3. Cell culture models replicate human FUS-opathies

HeLa cells expressing FUS with the P525L mutation demonstrated redistribution of FUS to the cytoplasm and, following heat shock, cytosolic FUS was sequestered into stress granules (Neumann et al., 2011). However, under these same conditions, there was no change in the subcellular distribution of TAF15 or EWS which remained localized to the nucleus and were not recruited into FUS-ir stress granules. These results mimic the findings in human cases of ALS-*FUS*. In contrast, when HeLa cells were transfected with the transportin-specific competitive inhibitor peptide M9M, all FET proteins showed redistribution to the cytoplasm with reduced nuclear localization. Following heat shock, all FET proteins co-localized within cytoplasmic stress granules, although the recruitment of EWS was somewhat less efficient. Thus, inhibition of transportin-mediated import in cultured cells replicates the situation of co-aggregation of all FET proteins seen in inclusions in FTLD-FUS.

4. Discussion

Based on our observations we hypothesize that ALS-*FUS* and FTLD-FUS arise from distinct pathomechanisms. Pathogenic *FUS* mutations usually alter the nuclear localization signal (Bosco et al., Dormann et al., 2010), resulting in a selective defect in FUS nuclear import with no involvement of other FET proteins. In FTLD-FUS, some more general defect in transportin-mediated nuclear import affects the cellular

distribution of all FET proteins. One possibility is that there is some abnormal post-translational modification of all FET proteins that interferes with their proper binding to transportin. Although we have not yet demonstrated the presence of abnormal M_r FET protein species in FTLD-FUS, the analysis of posttranslational modifications requires further investigation by more sensitive means, such as mass spectrometry.

Alternatively, a primary defect in transportin (as might arise from genetic variation in *TNPO1*) could reduce the efficiency of import, or a reduction in transportin expression could limit the availability of binding sites. However, against these latter mechanisms is preliminary data showing no alteration in the subcellular distribution of several other transportin cargos, such as hnRNPA1.

In summary, our findings add TAF15 and EWS to the list of RNA-binding proteins involved in neurodegeneration and make *TAF15* and *EWSR1*, as well as *TNPO1*, obvious candidate genes worthy of investigation in the FTD/ALS spectrum of disease (Couthouis et al. 2011, Ticozzi et al. 2011).. Although the respective roles of FUS, TAF15 and EWS dysregulation in FTLD remain to be elucidated, our findings suggest that a more appropriate designation for the FTLD molecular category that includes aFTLD-U, NIFID and BIBD might be FTLD-FET.

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Figure Caption

Figure 1 FUS-positive neuronal and glial inclusions in all FTLD-FUS subtypes are also immunoreactive for other FET proteins. **(a)** aFTLD-U, **(b)** BIBD, **(c)** NIFID.

Immunohistochemistry for TAF15 **(a and b)** and EWS **(c)**. Scale bar, 40 μ m.

Figure 1