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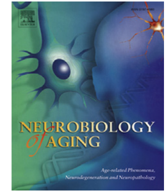

The presence of heterogeneous nuclear ribonucleoproteins in frontotemporal lobar degeneration with FUS-positive inclusions

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Frontotemporal lobar degeneration with fused in sarcoma–positive inclusions (FTLD-FUS) is a disease with unknown cause. Transportin 1 is abundantly found in FUS-positive inclusions and responsible for the nuclear import of the FET proteins of which FUS is a member. The presence of all FET proteins in pathological inclusions suggests a disturbance of transportin 1–mediated nuclear import. FUS also belongs to the heterogeneous nuclear ribonucleoprotein (hnRNP) protein family. We investigated whether hnRNP proteins are associated with FUS pathology implicating dysfunctional nuclear export in the pathogenesis of FTLD-FUS. hnRNP proteins were investigated in affected brain regions in FTLD-FUS using immunohistochemistry, biochemical analysis, and the expression analysis. We demonstrated the presence of several hnRNP proteins in pathological inclusions including neuronal cytoplasmic inclusions and dystrophic neurites. The biochemical analysis revealed a shift in the location of hnRNP A1 from the nucleus to the cytoplasm. The expression analysis revealed an increase in several hnRNP proteins in FTLD-FUS. These results implicate a wider dysregulation of movement between intracellular compartments, than mechanisms only affecting the nuclear import of FUS proteins.

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1. Introduction

Recent advances in our understanding of the molecular mechanisms associated with frontotemporal lobar degeneration have shown that this heterogeneous group of diseases can be divided on the presence of the abnormal protein aggregates found in the pathological inclusions (Mackenzie et al., 2010). Fused in sarcoma (FUS) is a protein identified in pathological inclusions of patients clinically characterized with frontotemporal dementia and pathologically termed frontotemporal lobar degeneration with fused in sarcoma–positive inclusions (FTLD-FUS) (Lashley et al., 2011; Munoz et al., 2009; Neumann et al., 2009)

It is still unknown how FUS plays a role in the pathogenesis of FTLD-FUS and which of its many known functions are disrupted that leads to the formation of pathological lesions. FUS is a multifunctional protein composed of 526 amino acids belonging to the FET family of proteins, which also includes Ewings sarcoma protein (EWS) and TATA-binding protein-associated factor 15 (TAF15) (Aman et al., 1996; Crozat et al., 1993; Kovar, 2011; Law et al., 2006; Yang et al., 2010). The FET family of proteins are all ubiquitously expressed nuclear proteins, which are highly conserved with predicted roles in RNA transcription, processing, transport, and DNA repair (Bertrand et al., 1999; Crozat et al., 1993; Kovar, 2011; Law et al., 2006; Perrotti et al., 1998). They are also found to shuttle between the nucleus and the cytoplasm, and their nuclear import is mediated by their nonclassical nuclear localization signal called PY-NLS, which is recognized by the nuclear import protein transportin 1 (TRN1). The pathological lesions in FTLD-FUS have been found to contain TRN1 (Brelstaff et al., 2011), EWS, and TAF15 in varying degrees (Davidson et al., 2013; Neumann et al., 2012), suggesting that the pathogenic mechanism in FTLD-FUS is related to the dysfunction of transportin-mediated nuclear import affecting all FET proteins that are transported by TRN1 (Neumann et al., 2012).

FUS is structurally characterized by an N-terminal serine, tyrosine, glycine, and glutamine-rich region, an RNA recognition motif, a C2-C2 zinc finger motif, multiple RGG repeat regions, and a nuclear localization signal (NLS) at the extreme C-terminus. The C-terminal region of FUS contains multiple domains involved in RNA-protein interactions, while the N-terminus is involved in transcription activation (Prasad et al., 1994). Due to its distinct structure and function, FUS also belongs to the heterogeneous nuclear ribonucleoproteins (hnRNPs) and is also known as hnRNP P2 (Calvio et al., 1995).

hnRNPs are a family of around 20 major polypeptides, hnRNPs A1–U, which range in size from 34 to 120 kDa (Pinol-Roma et al., 1988). Each protein contains at least 1 RNA-binding motif such as

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Table 1
Demographic data of cases with FUS-positive inclusions

Case number	Clinical diagnosis	Age of onset (y)	Age at death (y)	Disease duration (y)	Gender	PM delay (h)	pH
NIFID							
1	CBS	41	43	2	Female	55	5.78
2	bvFTD	43	46	3	Female	30	6.26
3	MND	44	46	2	Male	96	6.05
4	MND	63	68	5	Female	2	6.30
5	MND	69	72	3	Female	90	6.32
6	CBS	66	69	3	Female	101	n/a
aFTLD-U							
1	bvFTD	43	53	10	Female	96	n/a
2[a]	bvFTD	44	51	7	Male	24	6.63
3	bvFTD	47	52	5	Male	72	6.68
4	PSP	49	55	6	Female	3.5	6.30
5	bvFTD	51	60	9	Male	48	6.67
6[a]	bvFTD	55	58	3	Female	n/a	n/a
Normal controls							
1	Normal	n/a	88	n/a	Male	16	n/a
2	Normal	n/a	79	n/a	Female	89	n/a
3	Normal	n/a	80	n/a	Female	49	n/a
4	Normal	n/a	86	n/a	Female	120	n/a
5	Normal	n/a	93	n/a	Female	30	n/a
6	Normal	n/a	83	n/a	Female	99	n/a

Key: bvFTD, behavioural variant frontotemporal dementia; CBS, corticobasal syndrome; FUS, Fused in sarcoma; MND, motor neuron disease; NIFID, neuronal intermediate filament inclusion disease; PSP, progressive supranuclear palsy; PM, post-mortem delay.
[a] aFTLD-U2 (son) is related to aFTLD-U6 (mother).

an RNA recognition motif, a hnRNP K homology domain, or an arginine and/or glycine-rich box (Dreyfuss et al., 1993; Krecic and Swanson, 1999). Some hnRNPs contain auxiliary domains with unusual amino acid compositions, which mediate protein-protein interactions (Cartegni et al., 1996). Correlated with these diverse structural features a multitude of cellular functions have been ascribed to hnRNP proteins, including the roles in DNA maintenance, recombination, transcription, processing of primary transcripts, mRNA nuclear export, subcellular localization, translation, and stability of mature mRNA (Busch and Hertel, 2012; Dreyfuss et al., 1993, 2002; Roy et al., 2014). hnRNPs A1 and A2 constitute 60% of the total protein mass of hnRNP particles, representing the most abundant nuclear proteins (Beyer et al., 1977). These proteins are associated with pre-mRNAs in the nucleus and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport. While all hnRNPs are present in the nucleus, some shuttle between the nucleus and the cytoplasm and have distinct nucleic acid–binding properties. FUS, along with other hnRNP proteins, is exported from the nucleus, probably bound to mRNA and is immediately reimported once dissociated. Its M9 domain acts as both a nuclear localization and nuclear export signal (Macara, 2001; Xu and Massagué, 2004). However, FUS can be distinguished from other hnRNPs notably by the presence of an N-terminal peptide sequence that can serve as a transcriptional activation domain (Zinszner et al., 1994).

As FUS is a member of the hnRNP protein family, we wished to investigate whether any other hnRNP proteins were associated with FUS pathology and if they could be implicated in the pathogenesis of FTLD-FUS. We studied the localization of proteins of the hnRNP family in affected brain regions in patients with FTLD-FUS and normal control brains by immunohistochemistry, biochemical analysis, and investigated their expression using NanoString technology.

Table 2
Antibodies used in this study

Antibody	Antibody source	Cat. No	Specificity	Dilution	Antigen
FUS	Novus	NB100-565	Rb	1:200	aa 1–50
TAF15	Abcam	Ab69581	Rb	1:100	—
EWS	Santa Cruz	EWS-G5	Ms	1:200	N-terminal
hnRNP A2B1	Abcam	ab6102	Ms	1:100	—
hnRNP C1/C2	Abcam	ab97541	Rb	1:100	aa 1–152
hnRNP A1	Abcam	ab5832	Ms	1:100	Full length
hnRNP A1	Santa Cruz	sc-32301	Ms	1:200	C-terminal
hnRNP A1	Abcam	ab50492	Rb	1:100	N-terminal
hnRNP D1/D2	Abcam	ab61193	Rb	1:500	Phosphoserine 83
hnRNP E1/E2	Santa Cruz	sc-28725	Rb	1:100	aa 171–280
hnRNP F	Abcam	ab50982	Rb	1:100	aa 361–410
hnRNP G	Abcam	ab70064	Rb	1:200	N-terminus
hnRNP H	Abcam	ab10374	Rb	1:200	aa 400–448
hnRNP I	Santa Cruz	sc-16549	Gt	1:100	Internal sequence
hnRNP L	Abcam	ab6106	Ms	1:1000	Full length
hnRNP M	Sigma	HPA024344	Rb	1:1000	aa 344–448
hnRNP U	Abcam	ab10297	Ms	1:1000	Full length

Key: EWS, Ewings sarcoma protein; FUS, fused in sarcoma; hnRNP, heterogeneous nuclear ribonucleoprotein; TAF15, TATA-binding protein-associated factor 15.

2. Material and methods

2.1. Cases

Brains were donated to the Queen Square Brain Bank for Neurological Disorders, UCL Institute of Neurology, University College London; the MRC London Brain Bank for Neurodegenerative Diseases, Institute of Psychiatry, King's College, London, UK; Neuropathology Department, Århus Kommunehospital, Århus, Denmark; and NeuroResource, UCL Institute of Neurology, University College London. All cases had previously been diagnosed as neuronal intermediate filament inclusion disease (NIFID) (6 cases) or aFTLD-U (6 cases) characterized as having pathological inclusions that were immunoreactive for FUS and ubiquitin, but negative for both tau and TDP-43, with cases of the NIFID subgroup also containing α-internexin positive inclusions (Lashley et al., 2011). Ethical approval for the study was obtained from the National Hospital for Neurology and Neurosurgery Local Research Ethics Committee.

2.2. Immunohistochemistry

Seven-micron-thick tissue sections from the hippocampus, frontal cortex, and spinal cord where cut from the following cases listed in Table 1 (NIFID 1–6 and aFTLD-U 1–6) and 6 neurologically

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