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The Lonafarnib target, Rhes, is uniquely dysregulated in tauopathies: A human postmortem study

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Abstract

Background: The farnesyltransferase inhibitor, Lonafarnib, is capable of reducing tau lesions and associated atrophy in tauopathy models through activity on the Ras GTPase, Rhes (Hernandez I et al., 2019). While hinting at a role of Rhes in tau proteinopathic etiology, it is unclear how Rhes activity changes in human tauopathies or other neurodegenerative diseases. As these models reflect rare, familial forms of tauopathy, we sought to detect evidence of Rhes changes in human sporadic tauopathy versus FTLD-TDP and synucleinopathies.

Method: Allocortical and neocortical sections from postmortem cases (Table 1) representing five different tauopathies, FTLD-TDP, Lewy body disease, and healthy controls (HC) were immunohistochemically stained for Rhes, proteinopathic lesions (i.e. PHF-1 positive tau inclusions, phospho-TDP-43, or α-synuclein), and NeuN. Single-nucleus RNA-sequencing (snRNA-seq) was also performed in a separate cohort representing HC and early AD.

Result: 40 cases were examined in this study (Table 1). Rhes is widespread in cortical neurons, as detected by immunohistochemical staining and snRNA-seq (Figure 1). In tauopathies, neurons either present Rhes as diffuse or punctiform, or feature an absence of Rhes (Figure 2). Accordingly, we classified neurons as Rhes type Diffuse, Punctiform, or Absent. The proportion of neurons with PHF-1 positive tau inclusions significantly differ between the three neuronal Rhes phenotypes ($p < 0.0001$) with the majority of Absent type neurons harboring PHF-1 positive tau inclusions and Diffuse type neurons mostly devoid of PHF-1 positive tau inclusions (Figure 3, Table 2). Punctiform and Absent type neurons were not detected in neurons with phospho-TDP-43 inclusions or α-synuclein inclusions (Figure 4).

Conclusion: Given the distribution of PHF-1 positive tau inclusions across different Rhes-deliniated neuronal phenotypes, we propose a model by which Rhes takes on a punctiform phenotype then is ultimately cleared from neurons with tau lesions. This postmortem study in humans, together with in vivo and in vitro work done in familial tauopathy models, suggests the presence of a causal relationship between Rhes clearance and tau proteinopathic lesions, but not phospho-TDP-43 or α-synuclein inclusions. As such, Rhes cellular phenotype represents a novel postmortem hallmark of tauopathy and supports to application of Lonafarnib to the treatment of tauopathies.

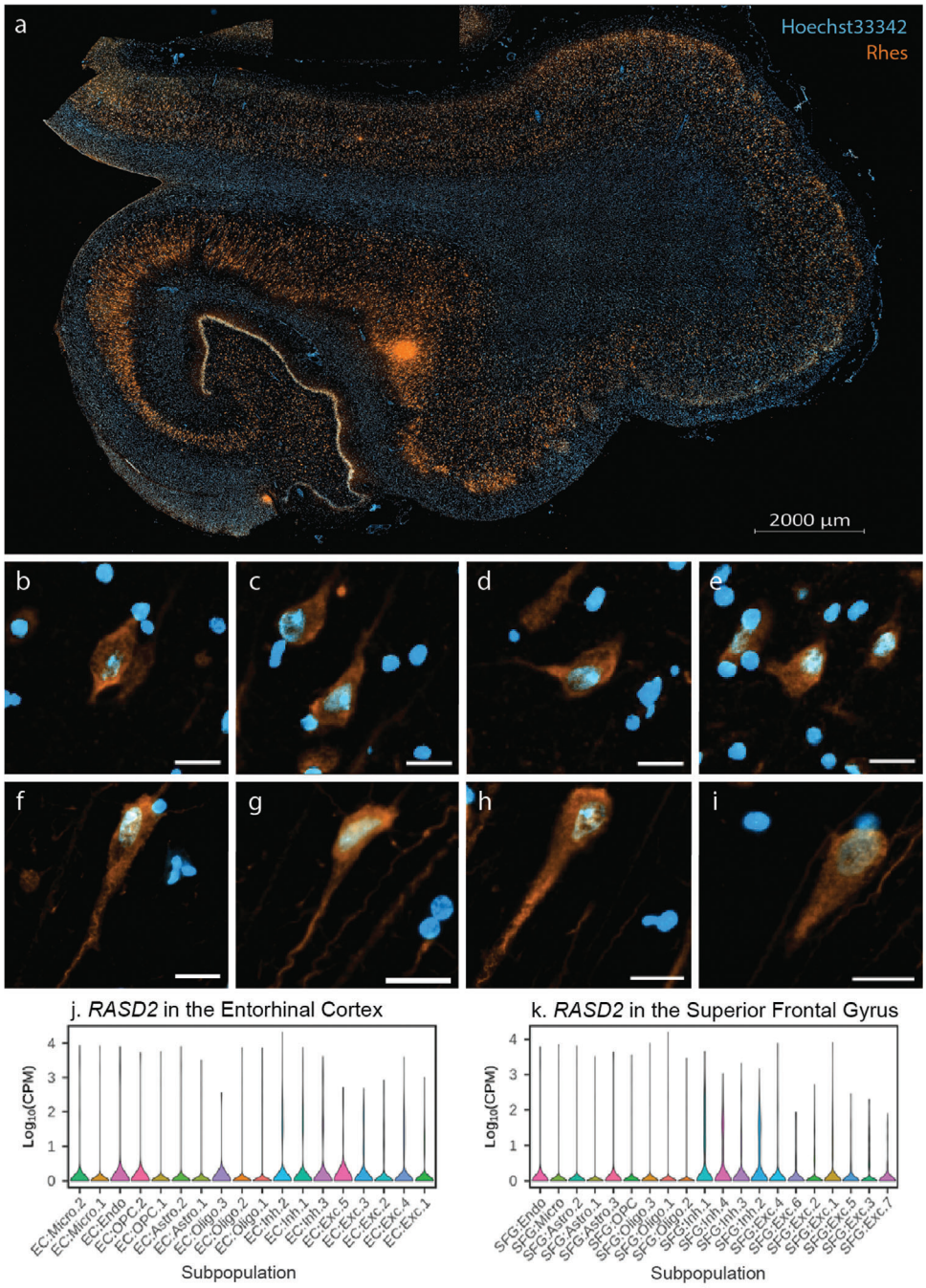

Figure 1: Rhes (orange) is ubiquitously expressed throughout the human hippocampal formation of an 82-year-old female free of any neuropathological diagnosis (a). See supplementary figure 1 for the overlapping NeuN distribution. In the entorhinal cortex (b-e) and cornu ammonis 1 (f-i) neurons, Rhes has a granular appearance in the cell body (Hoechst 33342 in blue), extending into proximal segments of neuronal processes (Type-I). All scale bars b-i correspond to 20 µm. (j-k) Violin plots of RASD2 transcript levels in different cell type subpopulations in the entorhinal cortex (EC) and superior frontal gyrus (SFG) from Leng et al. 2020. Cell type abbreviations: Exc, excitatory neurons; Oligo, oligodendrocytes; Astro, astrocytes; Inh, inhibitory neurons; OPC, oligodendrocyte precursor cells; Micro, microglia; Endo, endothelial cells.

FIGURE 1

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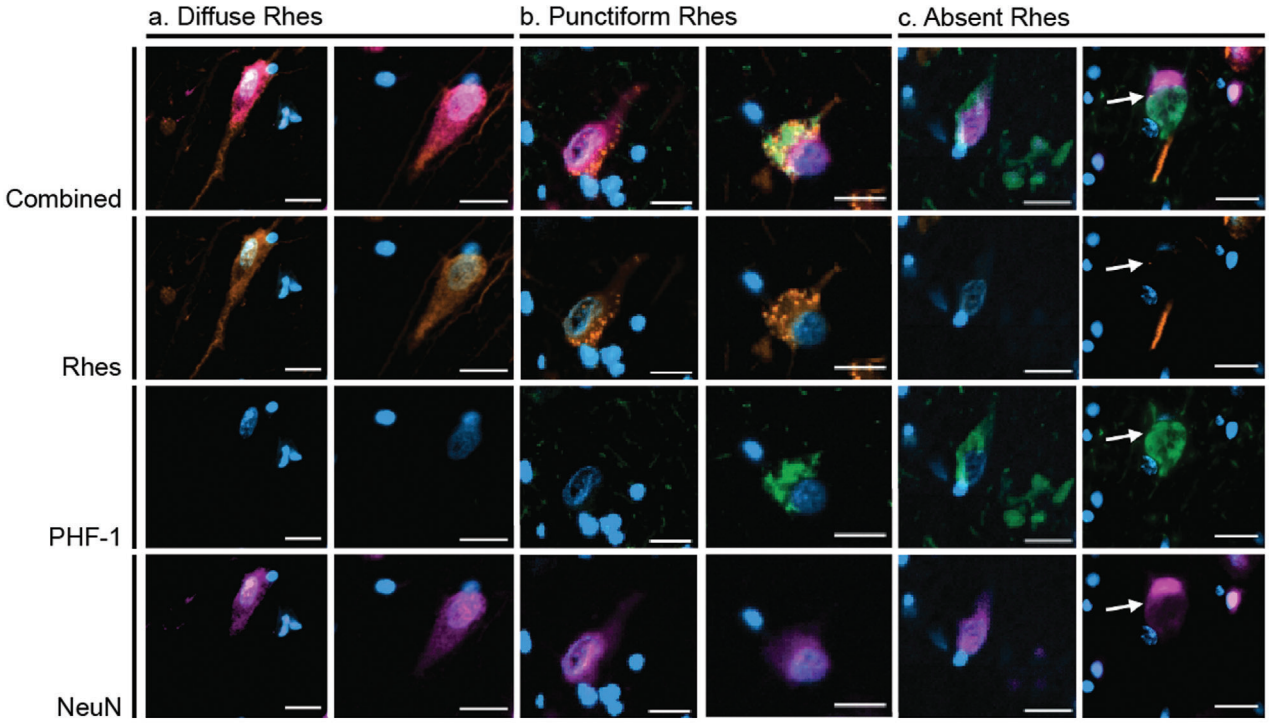

Figure 2: Diffuse Rhes (orange) neurons (a) feature diffuse Rhes staining in the cell body extending into the apical dendrite. Punctiform Rhes neurons (b) feature punctiform Rhes signal in the cell body and often co-occur with PHF-1 (pink) positive tau inclusions. Absent Rhes neurons (c) feature clearance of Rhes from the cell body and often co-occur with PHF-1 positive tau inclusions. Blue, DAPI; Orange, Rhes; Green, PHF-1; Pink, NeuN. All scale bars correspond to 20 μm.

FIGURE 2

TABLE 1

Table 1: Demographics and case characteristics for the quantitative and qualitative histological analyses in this study

Analysis	Diagnosis	n	Age (mean, sd)	Female (n, %)
Tauopathies (quantitative)	Healthy Controls	5	78, 10	5, 100%
	Alzheimer's disease	5	66, 5	2, 40%
	Argryophilic Grain Disease	5	84, 12	1, 20%
	Corticobasal Degeneration	5	66, 4	2, 40%
	Pick's Disease	5	68, 8	2, 40%
	Progressive Supranuclear Palsy	5	76, 10	3, 60%
	Total	30	73, 10	15, 50%
TDP-43 and synucleinopathy (qualitative)	FTLD-TDP Type A	3	63, 3.6	3, 100%
	FTLD-TDP Type B	4	59, 3.7	2, 50%
	Lewy Body Disease	3	77, 6.4	0, 0%

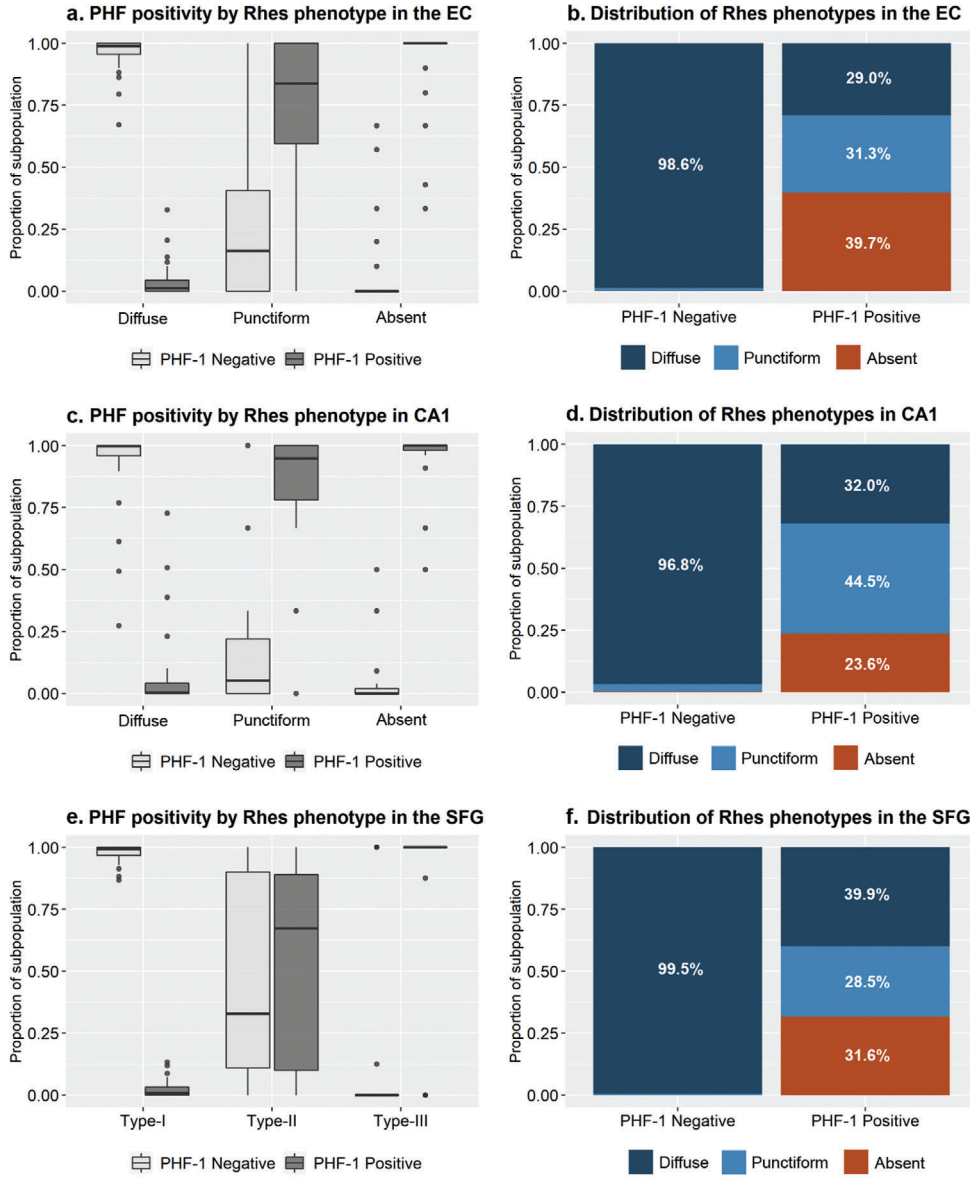

Figure 5: Entorhinal cortex neurons with diffuse Rhes are predominantly PHF-1 negative, while neurons with absent Rhes are predominantly PHF-1 positive (a). While neurons with punctiform Rhes appear to be roughly split between PHF-1 positive and negative neurons in the entorhinal cortex, they make up roughly a third of the PHF-1 positive neurons, indicating a tendency to co-occur with tau inclusions (b). Of the PHF-1 positive neurons in the entorhinal cortex, the majority have either punctiform or absent Rhes. This pattern largely holds for neurons of the cornu ammonis 1 and the superior frontal gyrus (c-f).

FIGURE 5

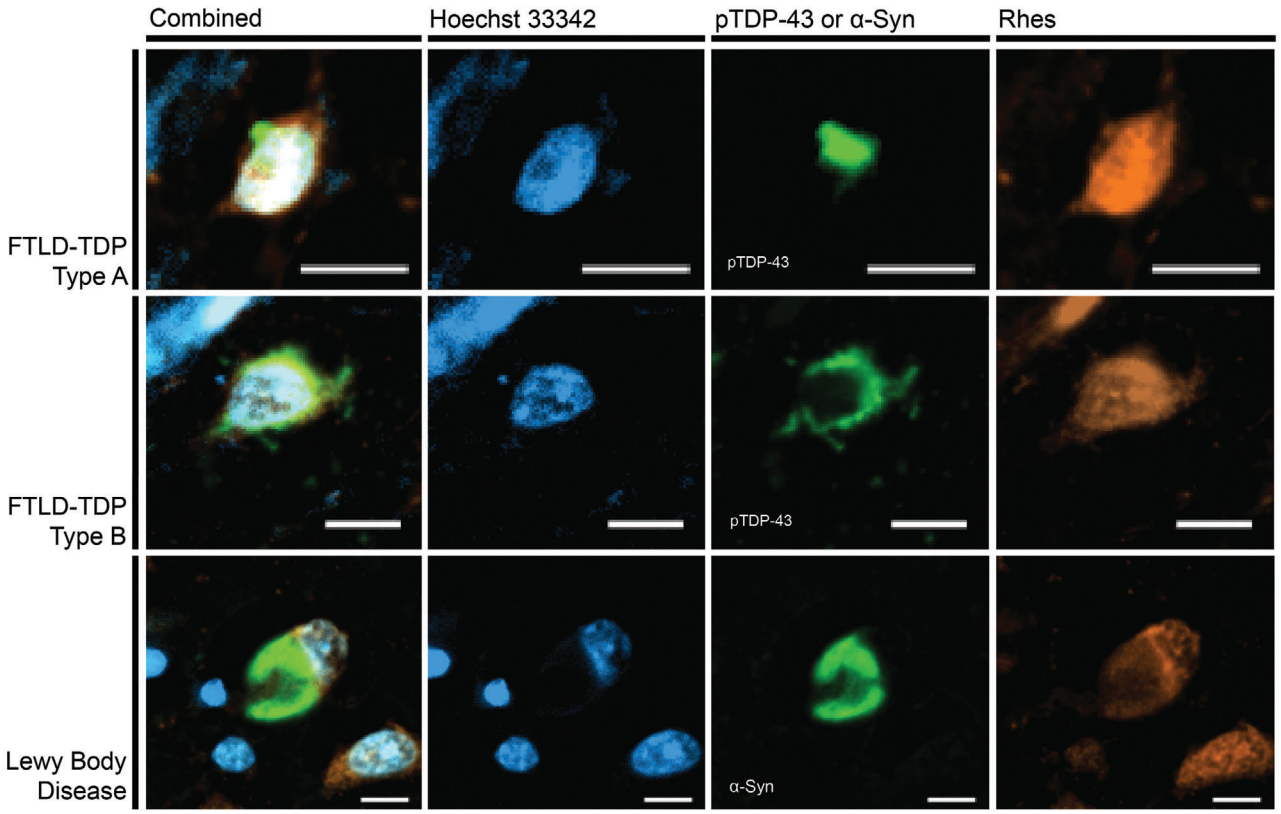

Figure 6: Neurons positive for pTDP-43 (green) proteinopathy display a Diffuse Rhes (orange) phenotype. The FTLD-TDP Type A images come from the inferior frontal gyrus of a 66-year-old female with behavioral variant frontotemporal dementia. The FTLD-TDP Type B images come from the middle frontal gyrus of a 57-year-old male with behavioral variant frontotemporal dementia and amyotrophic lateral sclerosis. The Lewy Body Disease images come from the anterior midcingulate cortex of a 70 year old male. Scale bar represents 10 μm.

FIGURE 6

TABLE 4

Table 4: Proportions (mean, sd) of each Rhes phenotype with PHF-1+ tau inclusions by region.

		Diffuse Rhes		Punctiform Rhes		Absent Rhes		
	Dx	PHF-1+	PHF-1-	PHF-1+	PHF-1-	PHF-1+	PHF-1-	p-value[a]
a) Entorhinal cortex	Total (n=30)	0.043, 0.073	0.957, 0.073	0.726, 0.323	0.274, 0.323	0.919, 0.188	0.081, 0.188	< 0.0001[b]
	HC (n=5)	0.01, 0.011	0.99, 0.011	0.556, 0.509	0.444, 0.509	0.889, 0.192	0.111, 0.192	0.0737
	AD (n=5)	0.074, 0.036	0.926, 0.036	0.688, 0.187	0.312, 0.187	0.96, 0.089	0.04, 0.089	0.0024
	AGD (n=5)	0.006, 0.009	0.994, 0.009	0.444, 0.509	0.556, 0.509	0.587, 0.361	0.413, 0.361	0.0693
	CBD (n=5)	0.004, 0.006	0.996, 0.006	0.675, 0.395	0.325, 0.395	1, 0	0, 0	0.0061
	PiD (n=5)	0.154, 0.118	0.846, 0.118	0.913, 0.092	0.087, 0.092	0.98, 0.045	0.02, 0.045	0.0049
	PSP (n=5)	0.008, 0.009	0.992, 0.009	0.927, 0.12	0.073, 0.12	1, 0	0, 0	0.0103
b) Cornu Ammonis 1	Total (n=30)	0.074, 0.171	0.926, 0.171	0.841, 0.25	0.159, 0.25	0.936, 0.149	0.064, 0.149	< 0.0001[b]
	HC (n=5)	0.01, 0.018	0.99, 0.018	0.508, 0.45	0.492, 0.45	1, 0	0, 0	0.0586
	AD (n=5)	0.067, 0.102	0.933, 0.102	0.833, 0.078	0.167, 0.078	0.992, 0.017	0.008, 0.017	0.0016
	AGD (n=5)	0.001, 0.003	0.999, 0.003	0.917, 0.167	0.083, 0.167	0.833, 0.289	0.167, 0.289	0.0107
	CBD (n=5)	0.011, 0.013	0.989, 0.013	1, 0	0, 0	NA[c]	NA[c]	0.0486
	PiD (n=5)	0.345, 0.294	0.655, 0.294	0.965, 0.052	0.035, 0.052	0.894, 0.157	0.106, 0.157	0.0145
	PSP (n=5)	0.011, 0.018	0.989, 0.018	0.778, 0.385	0.222, 0.385	1, 0	0, 0	0.0392
c) Superior frontal gyrus	Total (n=25)	0.028, 0.038	0.972, 0.038	0.567, 0.395	0.433, 0.395	0.851, 0.357	0.149, 0.357	< 0.0001[b]
	HC (n=5)	0, 0	1, 0	0, 0	1, 0	0, 0	1, 0	NA[d]
	AD (n=5)	0.056, 0.047	0.944, 0.047	0.764, 0.12	0.236, 0.12	0.8, 0.447	0.2, 0.447	0.0365
	CBD (n=5)	0.014, 0.013	0.986, 0.013	0.413, 0.433	0.587, 0.433	0.775, 0.437	0.225, 0.437	0.1765
	PiD (n=5)	0.065, 0.043	0.935, 0.043	0.816, 0.215	0.184, 0.215	1, 0	0, 0	0.0023
	PSP (n=5)	0.005, 0.003	0.995, 0.003	0.625, 0.479	0.375, 0.479	1, 0	0, 0	0.0163

[a]*p-value computed by a Kruskal-Wallis rank sum test comparing the PHF-1+ proportions in each Rhes phenotype for each disease.*
[b]*p-value for all diseases combined computed using a one-way ANOVA comparing the PHF-1+ proportions in each Rhes phenotype*
[c]*No CA1 neurons with the absent Rhes phenotype were detected in CBD cases*
[d]*The Kruskal-Wallis rank sum test cannot estimate the significant of data lacking variance*
Abbreviations: HC, Healthy Controls; AD, Alzheimer's disease; AGD, Argyrophilic Grain Disease; CBD, Corticobasal Degeneration; PiD, Pick's disease; PSP, Progressive Supranuclear Palsy