JEM

SUPPLEMENTAL MATERIAL

Gomez-Sanchez et al., http://www.jem.org/cgi/content/full/jem.20101523/DC1

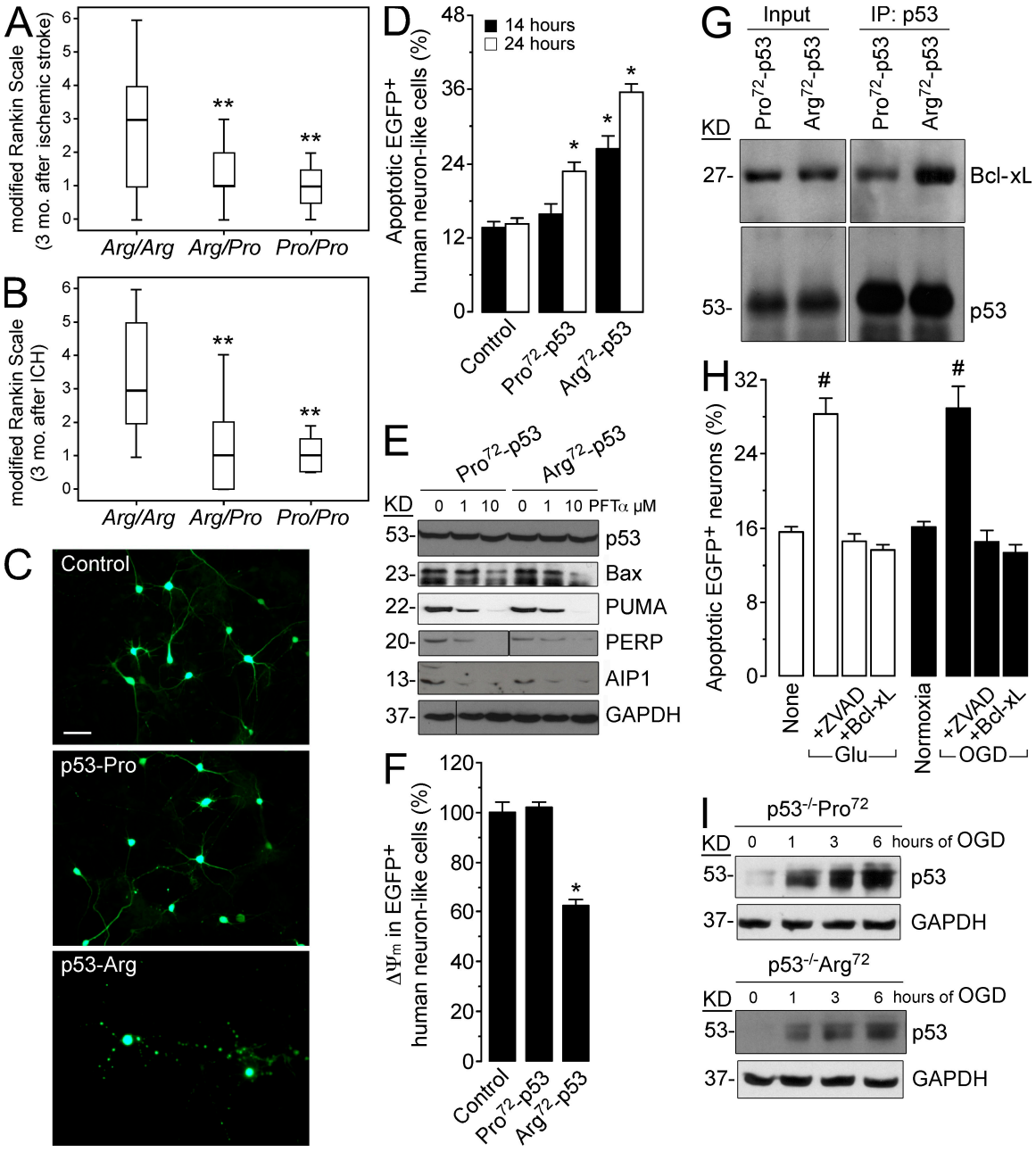

Figure S1. Arg72-p53 dictates poor prognosis after both ischemic stroke and intracerebral hemorrhage in the validation cohort and promotes apoptosis through the intrinsic pathway in different cell types. (A and B) Patients were admitted at the University Hospital of Gremans Trias I Pujol (Catalonia, Spain). The study included 103 (*Arg/Arg*: 61; *Arg/Pro*: 35; *Pro/Pro*: 7) patients with ischemic stroke (A) and 48 (*Arg/Arg*: 30; *Arg/Pro*: 16; *Pro/Pro*: 2) patients with ICH (B). Scores of the mRS were measured in patients with indicated Tp53 codon 72 genotypes. (C) Rat cortical primary neurons were transfected with Arg^{72}-p53-IRES-EGFP or Pro^{72}-p53-IRES-EGFP. Fluorescence microphotographs were taken 14 h later. (D) Terminally differentiated human neuron-like cells were transfected with empty vector (control) or vector encoding Arg^{72}-p53-IRES-EGFP or Pro^{72}-p53-IRES-EGFP. Apoptosis of $EGFP^+$ cells was measured 14 or 24 h later. (E) HEK293T cells were transfected with empty vector (control) or vector encoding Arg^{72}-p53 or Pro^{72}-p53 and were treated with the inhibitor of p53-mediated gene transactivation, PFT-α. Proteins were measured by Western blotting 14 h later. (F) $\Delta\psi_m$ was measured in terminally differentiated human neuron-like cells transfected as described in D. (G) HEK293T cells were transfected vector encoding Arg^{72}-p53 or Pro^{72}-p53. p53 was immunoprecipitated with Bcl-xL and proteins were analyzed by Western blotting. (H) Neurons were transfected with the minimum amount of Arg^{72}-p53-IRES-EGFP cDNA not altering neuronal survival (0.08 μg/10^6 neurons) and were exposed to 100 μM glutamate (Glu) for 5 min and further incubated in culture medium for 8 h or to oxygen and glucose deprivation for 1 h (OGD). Neurons were treated with 100 μM of the caspase inhibitor ZVAD or cotransfected with Bcl-xL. Apoptosis was measured. (I) p53-null H1299 cells were transfected with human BAC containing the entire *Tp53* gene locus encoding either Pro ($p53^{-/-}Pro^{72}$) or Arg ($p53^{-/-}Arg^{72}$) and were exposed to OGD. Proteins were analyzed by Western blotting. The data in C, E, G, and I represent four independent experiments. The data in D, F, and H are means ± SEM of four different cell cultures. **, $P < 0.001$ compared with *Arg/Arg* genotype; *, $P < 0.05$ compared with Pro^{72}-p53; #, $P < 0.05$ compared with none or normoxia in H. Bar, 50 μM.

Table S1. Baseline characteristics of patients

Characteristic	Ischemic stroke		ICH	
	Cohort 1 *n* = 408	Cohort 2 *n* = 103	Cohort 1 *n* = 128	Cohort 2 *n* = 47
Age, years	72.8 (12.1)	67.4 (10.1)	71.2 (12.3)	67.1 (12.7)
Males, %	59.3	68.9	57.8	76.6
NIHSS on admission	5 [2, 11]	4 [2, 8]	8 [4, 12]	11 [6, 16]
Infarct volume, ml	31.1 (58.7)	25.4 (48.9)		
ICH volume, ml			20.5 (26.9)	20.5 (20.3)
END, %	10.5	6.8		
Poor prognosis at 3 mo, %	39.5	33.0	46.1	55.3
TOAST:				
-Cardioembolic, %	36.3	41.7		
-Atherothrombotic, %	9.3	18.4		
-Lacunar, %	11.3	12.6		
-Undetermined, %	40.9	24.4		
-Others, %	2.2	2.9		
Etiology:				
-Hypertensive, %			59.4	72.3
-Anticoagulant, %			6.2	6.4
-Amyloid, %			14.1	4.3
-Undetermined, %			18.8	17.0
Tp53 Arg72Pro polymorphism:				
-*Arg/Arg* genotype, %	57.6	59.3	52.3	63.8
-*Arg/Pro* genotype, %	36.0	34.4	42.2	34.0
-*Pro/Pro* genotype, %	6.4	6.3	5.5	2.1
Tp53 Ins16bp polymorphism:				
-*A1/A1* genotype, %	71.6	74.0	73.4	80.2
-*A1/A2* genotype, %	27.2	24.0	23.7	15.7
-*A2/A2* genotype, %	1.2	1.9	2.9	2.1

Data are numbers (%), means (SD), or medians [quartiles]. INR, international normalized ratio; *A1*: no duplication; *A2*: 16 bp duplication. Cohort 1: patients admitted at the University Hospital of Santiago de Compostela (Galicia, Spain). Cohort 2: patients admitted at the University Hospital Germans Trias i Pujol (Catalonia, Spain).

Table S2. Univariate analysis of variables according to prognosis in stroke patients

Variable	Ischemic stroke			ICH		
	Good prognosis *n* = 69	Poor prognosis *n* = 34	P-value	Good prognosis *n* = 22	Poor prognosis *n* = 26	P-value
Age, years	68.3 (9.0)	70.0 (11.0)	0.024	60.1 (13.6)	71.7 (11.0)	0.005
Males, %	69.6	67.6	0.507	56.5	59.3	0.390
Hypertension, %	64.2	70.6	0.339	55.1	55.9	0.422
Diabetes, %	23.5	33.3	0.209	10.1	28.8	0.539
Smoking, %	21.7	23.5	0.512	10.1	11.9	0.535
Heavy alcohol intake, %	15.9	17.6	0.516	17.4	11.9	0.549
Hyperlipidemia, %	39.7	48.5	0.266			
Coronary disease, %	38.2	38.2	0.588	5.8	10.2	0.574
Atrial fibrillation, %	14.7	24.8	0.403	15.9	10.2	0.610
SBP, mm Hg	154.8 (22.6)	156.4 (37.3)	0.218	154.5 (30.2)	165.9 (28.7)	0.542
DBP, mm Hg	78.9 (13.1)	84.4 (15.8)	0.483	88.6 (17.8)	87.6 (16.8)	0.314
Temperature, °C	36.1 (0.5)	36.1 (0.7)	0.334	36.3 (0.5)	36. (0.8)	0.408
Bood glucose, mg/dL	131.4 (50.1)	156.1 (82.7)	0.240	114.5 (28.5)	134.8 (41.8)	0.132
Leukocytes, $\times 10^3$/ml	9.0 (1.8)	9.4 (4.7)	0.252	8.3 (2.3)	9.4 (3.3)	0.120
Platelet number, $\times 10^3$/ml	252.3 (66.3)	243,4 (64.3)	0.496	219.8 (52.7)	246.6 (117.1)	0.725
INR	87.7 (14.7)	90.5 (15.5)	0.260	93.1 (18.9)	91.6 (18.4)	0.900
Fibrinogen, mg/dL	510.6 (117.7)	542.1 (170.7)	0.751	453.8 (120.5)	504.6 (113.7)	0.448
NIHSS	3 [2, 6]	9 [5, 16]	<0.0001	3 [2,6]	10 [8,15]	<0.0001
END, %	2.9	14.7	0.038			
Infarct Volume, ml	7.4 (12.4)	43.4 (54.7)	<0.0001			
TOAST			0.148			
ICH volume on admission, ml				11.2 (11.4)	27.5 (37.8)	<0.0001
Edema volume, ml				7.5 (12.9)	28.8 (36.0)	<0.0001
Etiology						0.391
Tp53 Arg72Pro polymorphism			0.007			0.004
- *Arg/Arg genotype*, %	50.7	76.5		38.1	84.6	
- *Arg/Pro genotype*, %	39.1	23.5		57.1	15.4	
- *Pro/Pro genotype*, %	10.1	0		4.8	0	

Patients were admitted at the University Hospital of Germans Trias i Pujol (Catalonia, Spain; cohort 2). Functional outcome was evaluated at 3 mo using the mRS. An mRS score >2 was considered poor prognosis. Data are numbers (%), means (SD), or medians [quartiles]. Data were compared among prognosis groups (goor prognosis vs. poor prognosis) using the χ^2 test (proportions), the Student's *t* test (continuous variables with normal distribution), or the Mann-Whitney test (continuous variables without normal distribution, NIHSS). SBP, systolic blood pressure; DBP, diastolic blood pressure; INR, international normalized ratio. Univariate analysis of variables from cohort 1 (Galicia, Spain) is shown in Table II.

Table S3. Logistic regression analysis showing independent variables associated with poor functional outcome at 3 mo (mRS >2) after stroke

Variable	Ischemic stroke			ICH		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.08	1.00–1.17	0.029	1.08	0.99–1.17	0.059
ICH volume				0.98	0.93–1.04	0.677
NIHSS on admission	1.12	0.99–1.26	0.071	1.25	1.03–1.51	0.023
Infarct volume	1.03	1.00–1.06	0.011			
END	23.80	2.75–205.75	0.040			
Arg/Arg genotype	3.40	1.12–11.80	0.024	4.73	1.17–29.78	0.013

Patients were admitted at the University Hospital of Germans Trias i Pujol (Catalonia, Spain; cohort 2). OR and their 95% CI were calculated to demonstrate the independent association between poor prognosis and *Arg/Arg* genotype. Analysis of variables from cohort 1 (Galicia, Spain) is shown in Table III.

Table S4. *Tp53 Arg72Pro genotypes* in stroke patients with poor prognosis matched for infarct volume

Infarct volume	Poor prognosis (3 mo after ischemic stroke)		
	n	*Arg/Arg* genotype	*Arg/Pro* + *Pro/Pro* genotypes
ml			
0–2	112	17/52 (32.7%)	3/60 (5.0%)
2–10	91	13/44 (29.5%)	2/47 (4.2%)
10–28	96	23/54 (42.6%)	10/42 (23.8%)
>28	100	70/77 (90.9%)	15/23 (65.3%)

Patients were admitted at the University Hospital of Santiago de Compostela (Galicia, Spain). Functional outcome was evaluated at 3 mo using the mRS; mRS score >2 was considered poor prognosis. Data were compared among genotype groups using the χ^2 test ($P < 0.0001$).

Table S5. mRS

Grade	Criteria
0	No symptoms at all
1	No significant disability: despite symptoms, able to carry out all usual duties and activities
2	Slight disability: unable to perform all previous activities but able to look after own affairs without assistance
3	Moderate disability: requiring some help but able to walk without assistance
4	Moderately severe disability: unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability: bedridden, incontinent and requiring constant nursing care and attention
6	Death

Modified from Banks and Marotta (2007. *Stroke*. doi:10.1161/01.STR.0000258355.23810.c6).