JM17 for Treatment of Spinal-bulbar Muscular Atrophy and Neurodegenerative Disorders

JM17作為甘迺迪式症與神經退化性疾病的新療法

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ABSTRACT

Spinal-bulbar muscular atrophy (SBMA) is an inherited genetic neuromuscular disorder caused by abnormal aggregation of polyQ-expanded androgen receptor (AR). Currently, there is no effective or approved treatment for SBMA. JM17, a novel Nrf2 activator, has been shown to mitigate SBMA phenotypes in a mouse model by promoting AR degradation mediated by Nrf1 and Nrf2 pathways. JM17 displayed a higher potency in activating Nrf2-mediated antioxidant genes compared to DMF, a well-known Nrf2 activator approved for multiple sclerosis. Our study suggested a mechanism of JM17 disrupting Nrf2 interaction with its key regulator Keap1 to increase the stability of Nrf2 protein. RNA sequencing study in SBMA fibroblast confirmed a transcriptome signature consistent with Nrf1 and Nrf2 activation in expression of proteasome subunits and antioxidant enzymes, respectively. Moreover, JM17 was shown to protect neuronal N2a cells from H_2O_2-induced challenge; reduce apoptosis of the cells with defective mitochondrial respiratory chain; suppress pro-inflammatory cytokines, TNF-α, IL-1β, and IL-17a in mouse bone-marrow derived macrophage splenocytes efficiently; inhibit activation of human T-cells and human brain-derived microglia cell line. Altogether, these results suggested JM17 has pleiotropic activities and potential for treating other neurological disorders associated with oxidative stress and inflammation. A first-in-human Phase I study revealed an excellent safety and pharmacokinetic profile of JM17. Enrollment of the first-in-patient trial will be initiated by the end of 2022 to assess the safety of JM17 and its pharmacodynamic effects in muscles.

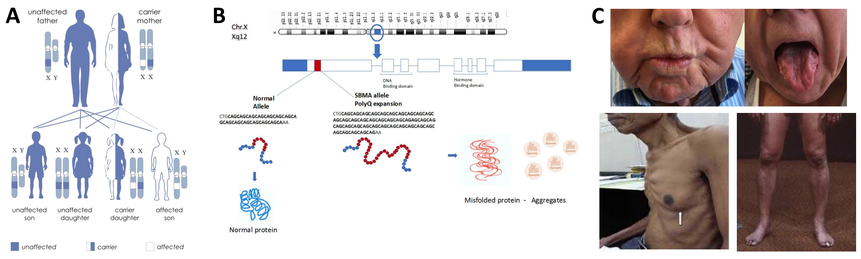


Figure 1. The introduction of SBMA. (A) SBMA, also referred to as Kennedy's disease, is a X-link genetic inherited disease, which almost exclusively affects male. (B) PolyQ expansion in androgen receptor (AR) causes AR aggregation and leads to spinal-bulbar muscular atrophy. (C) The bulbar muscle involvement affects chewing, speech and swallowing, which leads to choking or inhaling foods or liquids, and results in airway infection. SBMA also involves weakness and atrophy of limbs muscle, leading to difficulty walking and injury caused by falling.

JM17 is an effective Nrf2 activator

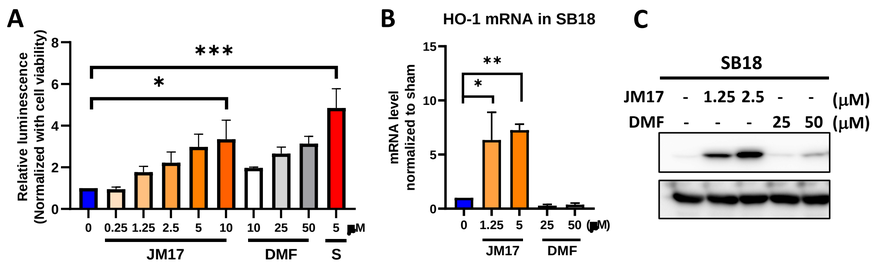


Figure 2. The comparison of JM17 and DMF in activating Nrf2. (A) The Nrf2 stability was monitored with HEK293 cells expressing Nrf2::Luciferase. The intensity of luminescence was detected after the cells were incubated with JM17, dimethyl fumarate (DMF) for 4 hours. Sulforaphane (S) was used as a positive control. (B, C) SB18, fibroblast from SBMA patient, was incubated with JM17 or DMF at indicated concentrations for 24 hours. The mRNA and protein levels of a Nrf2-mediated gene, Heme Oxygenase-1 (HO-1), was monitored. Data are expressed as mean ± SEM; unpaired two-tailed Student's t-test; **p* <0.05; ***p* <0.01; ****p* <0.001

JM17 disrupts the binding of KEAP1 to Nrf2

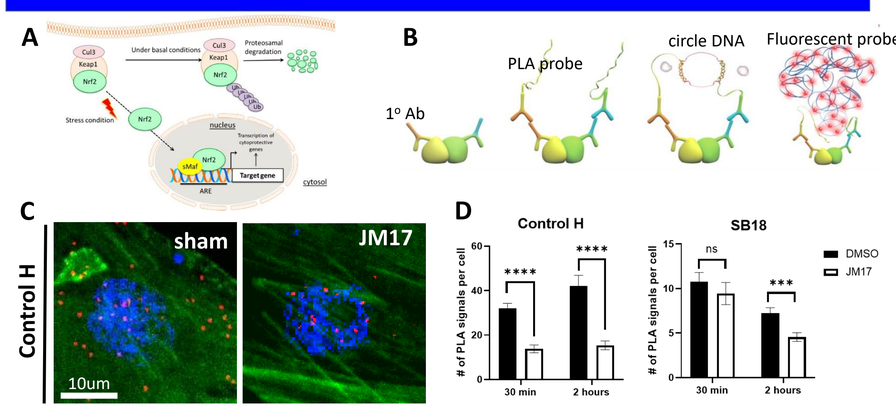


Figure 3. Assessing the physical interaction of Keap1 with Nrf2 by using PLA assay. (A) Keap1 is the negative regulator of Nrf2 by physically binding to Nrf2. Under basal condition, Keap1 triggers ubiquitination of Nrf2 and results in Nrf2 degradation. Under stress condition, Keap1-Nrf2 interaction is disrupted, releasing the free-form Nrf2 and triggering the expression of Nrf2-mediated antioxidant genes, like HO-1 (Heme oxygenase-1). (B) Proximity ligation assay (PLA) assay allows the detection of protein-protein interaction. Two primary antibodies label the proteins of interest, respectively. 2° antibodies containing PLA probes bind to primary antibodies. If the PLA probes are in proximity, the DNA strands on the probe can form a rolling circle DNA and trigger DNA synthesis chain reaction. Additional fluorescent-labeled probes are added and bind to the amplified DNA, which indicates the proximity. (C) The representative images of the PLA signals (red) in Control H cells with or without JM17 treatment. Green is actin labeled by phalloidin. (D) The PLA signals significantly reduced in both Control H and SB18 cells after treatment with JM17 for 30 minutes or 2 hours. Data are expressed as mean ± SEM; unpaired two-tailed Student's t-test; ****p* <0.001; *****p* <0.0001; ns= not significant

JM17 activates Nrf2 pathway, proteasome subunits and heat-shock proteins in SBMA fibroblast

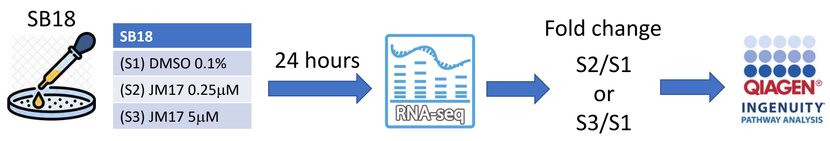


Figure 4. The scheme of RNA-sequencing study in SBMA fibroblast treated with or without JM17. SB18, fibroblast from SBMA patient, was collected and incubated with DMSO, 0.25μM JM17 or 5μM JM17 for 24 hours. The cells were then collected for RNA sequencing and the data was analyzed by using QIAGEN Ingenuity Pathway Analysis (IPA®).

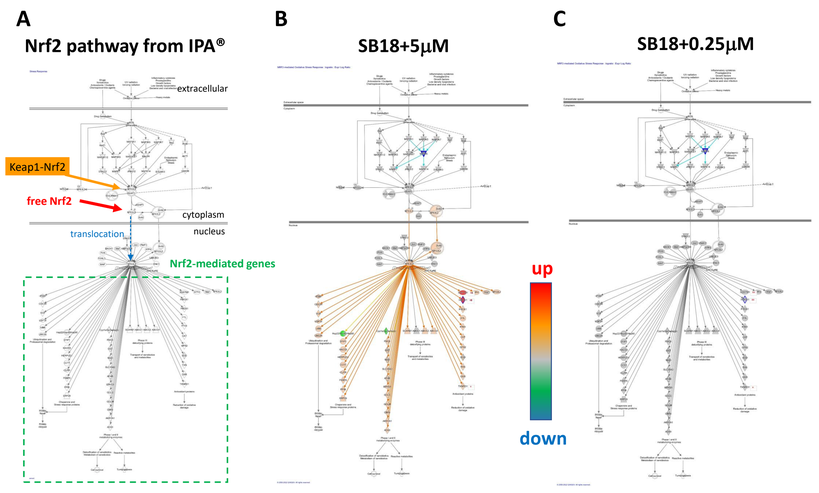


Figure 5. The effect of JM17 on Nrf2 pathway. (A) The Nrf2 pathway from IPA® shows the Keap1-Nrf2 complex (orange), free-form Nrf2 (red), the translocation of Nrf2 (blue) and the Nrf2-mediated genes (green. (B, C) The fold changes of RNA levels in SB18 after treatments were analyzed by using QIAGEN Ingenuity Pathway Analysis (IPA®). Orange/Red: upregulated; blue/green: downregulated; grey: no significant change.

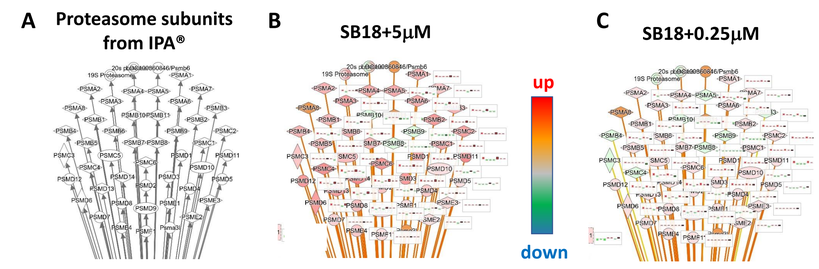


Figure 6. The effect of JM17 on the subunits of 26S proteasome. (A) The overview of the genes of 26S proteasome from IPA®. (B, C) The fold changes of mRNA levels after treatment with JM17 at 5μM (B) or 0.25μM (C) were demonstrated by IPA®. Orange/Red: upregulated; blue/green: downregulated; grey: no significant change.

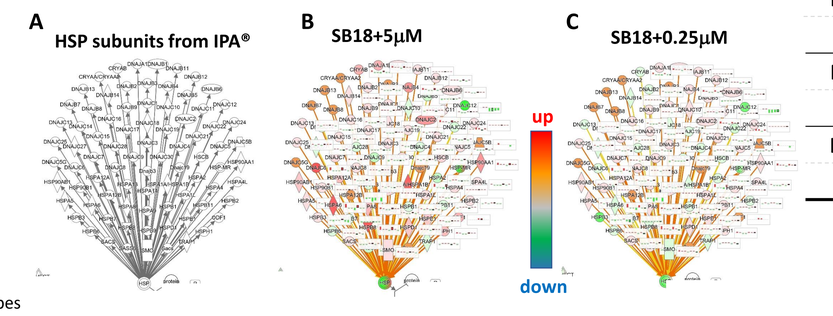


Figure 7. The effect of JM17 on the subunits of heat-shock proteins (HSP). (A) The overview of HSP genes from IPA®. (B, C) The fold changes of HSP mRNA levels after treatment with JM17 at 5μM (B) or 0.25μM (C) were demonstrated by IPA®. Orange/Red: upregulated; blue/green: downregulated; grey: no significant change.

JM17 protects neurons from H_2O_2-induced cell death

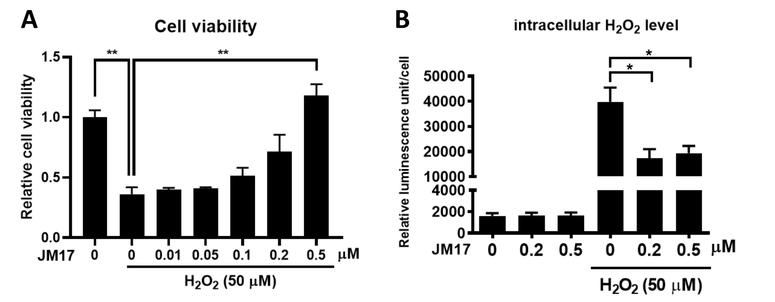


Figure 8. The effect of JM17 on the cell death and intracellular H_2O_2 levels of neuronal N2a cells under H_2O_2 challenge . The differentiated N2a cells were incubated with 50μM H_2O_2 together with JM17 at concentrations indicated for 24 hours. (A) The cell viability was assessed with MTT assay. (B) The intracellular H_2O_2 levels were assessed by ROS-Glo H_2O_2 assay. Data are expressed as mean ± SD; unpaired two-tailed Student's t-test; **p* <0.05; ***p* <0.01.

JM17 suppresses pro-inflammatory cytokines and chemokines

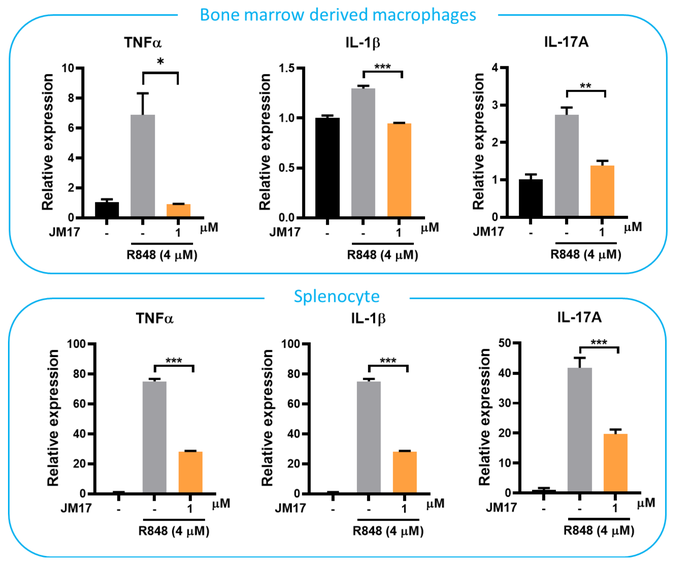
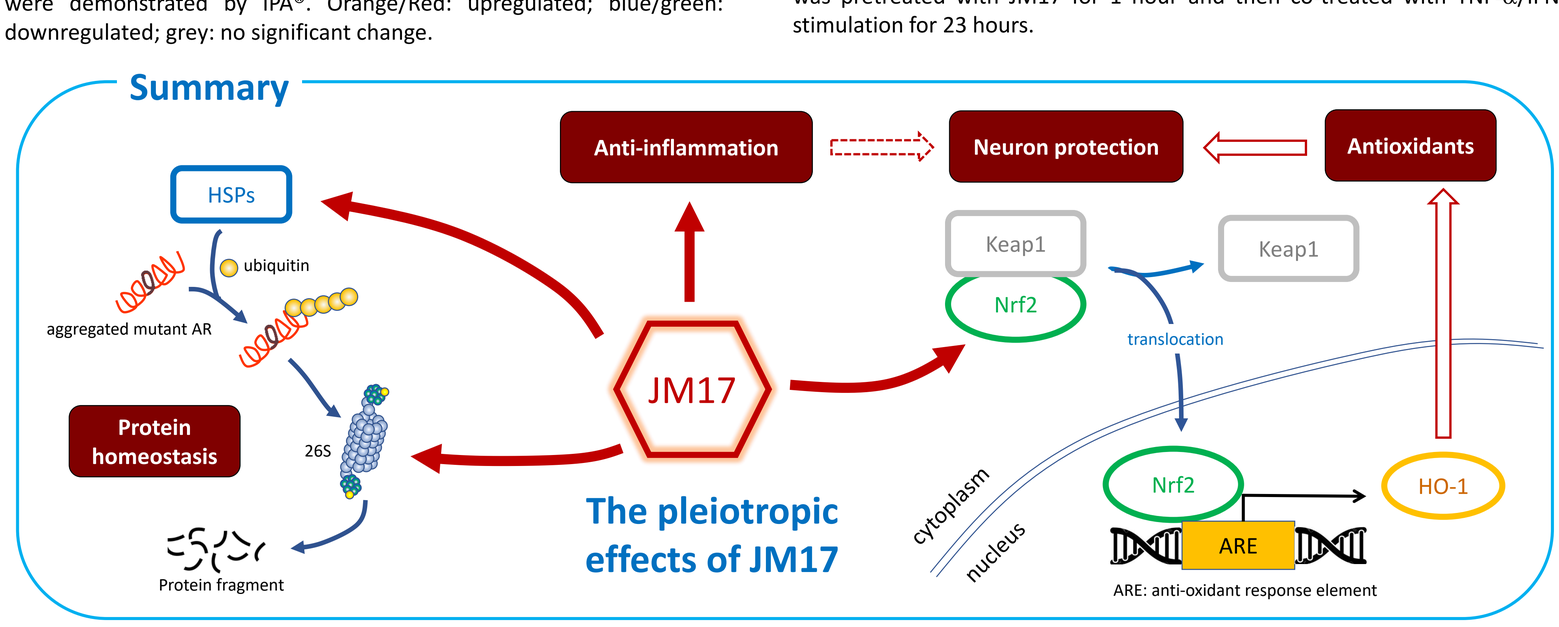


Figure 9. The suppression of JM17 in R848-stimulated BMDM and splenocytes . Mouse bone marrow derived macrophages (BMDM) or splenocytes were incubated with JM17 and R848 (Resiquimod) for 24 hours. The expression of TNFα, IL-1β and IL17A were measured by RT-qPCR. Data are expressed as mean ± SEM; unpaired two-tailed Student's t-test; **p* <0.05; ***p* <0.01; ****p* <0.001.

Summary Table of JM17 IC_{50} (mean ± SD; μM)

PBMC:	GM-CSF	IFN-g	IL-1b	IL-2	IL-6	IL-8
IC_{50}	0.67 ± 0.41	0.35 ± 0.05	0.26 ± 0.15	0.4 ± 0.19	0.22 ± 0.17	0.88 ± 0.45
PBMC:	IL-10	IL-17A	IL-21	MIP-1a	TNF-a	
IC_{50}	0.27 ± 0.12	0.49 ± 0.31	0.05 ± 0.47	0.75 ± 0.35	0.55 ± 0.26	
HMC3:	CXCL10	IL-8	IL-6	HLA-ABC		
IC_{50}	0.98 ± 0.04	1.05 ± 0.05	0.97 ± 0.01	1.00 ± 0.01		

Table 1. IC_{50} of JM17 for suppression of proinflammatory cytokines and chemokines in human PBMC and HMC3 cells. For PBMCs (peripheral blood mononuclear cells) assay, PBMCs were isolated from three human donors, and pre-treated with JM17 for 1 hour, followed by co-treatment with CD3-stimulation for 48 hours. The supernatants were then collected for further analysis. For HMC3 (human microglia cell line, ATCC® CRL-3304™) assay, HMC3 was pretreated with JM17 for 1 hour and then co-treated with TNF-α/IFN-γ-stimulation for 23 hours.



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REFERENCES

Figure 1A: Breza and Koutisis. J Nrufol. 2019;266(3):565
Figure 3A: Panieri et al., Antioxidants (Basel). 2020;9(3):193