Supplementary Information

RASSF7 negatively regulates pro-apoptotic JNK signaling by inhibiting the activity of phosphorylated-MKK7

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Supplementary Materials and Methods

HaCaT cell culture. HaCaT cells were maintained in the same way as HeLa or 293T cells.

RNAi. Sequence of RASSF7 #2 25-nucleotide oligomers (134-158 nt) was as follows; 5′-GGUCAUCGCACUAGCCCAAGCAAUA-3′/5′-UAUUGCUUGGGCUAGUGCGA UGACC-3′.

Supplementary Figures

Supplementary Figure S1 Effects of SP600125 on UV-induced JNK and p38 activation in control and RASSF7-knockdown cells. HeLa cells treated with control or RASSF7 siRNA were pre-treated for 1 h without (-) or with SP600125 (20 μM). Cells were then exposed to UV irradiation (100 J/m^2) and cultured for the indicated times. Lysates were immunoblotted to detect the indicated proteins.

Supplementary Figure S2 Effects of another RASSF7-siRNA in HeLa cells (**a**) Sequences of RASSF7 siRNAs. (**b**) Validation of RASSF7 siRNA #2. HeLa cells were treated with control or RASSF7 siRNA #2. Lysates were immunoblotted to detect RASSF7 and GAPDH (loading control). (**c**) Increased p-JNK but unaltered p-p38. HeLa cells treated with control or RASSF7 siRNA #2 were exposed to UV (100 J/m^2) and cultured for the indicated times. Lysates were immunoblotted (top) to detect total and phosphorylated JNK (p-JNK), and total and phosphorylated p38 (p-p38). GADPH, loading control. (**d**) Quantitation of increased caspase-3 cleavage and specificity for JNK. HeLa cells treated with control or RASSF7 siRNA #2 were further treated with DMSO (control) or 20 μM SP600125 (JNK inhibitor) and cultured for 1 h. Cells positive for cleaved caspase-3 were quantitated as for Figure 1**c** and **d**. Values shown are the mean ± s.d. **P*<0.05, compared to control siRNA.

Supplementary Figure S3 Effects of RASSF7-knockdown in HaCaT cells (**a**) Validation of RASSF7 siRNA. HaCaT cells were treated with control or RASSF7 siRNA. Lysates were immunoblotted to detect RASSF7 and GAPDH (loading control). (**b**) Increased cleavage of apoptotic mediators. HaCaT cells treated with control or RASSF7 siRNA were exposed to UV irradiation (100 J/m^2) and cultured for indicated times. Lysates were immunoblotted to detect the indicated proteins. GADPH, loading control. (**c**) Increased p-JNK but unaltered p-p38. HaCaT cells treated with control or RASSF7 siRNA were exposed to UV (100 J/m^2) and cultured for the indicated times.

Lysates were immunoblotted (top) to detect total and phosphorylated JNK (p-JNK), and total and phosphorylated p38 (p-p38). GADPH, loading control.

Supplementary Figure S4 Non-classical structural features of RASSF7. (**a**) Schematic representation of the protein domains of RASSF1 (NP_009113), RASSF2 (NP_739580), RASSF3 (NP_835463), RASSF4 (NP_114412), RASSF5/NORE1A (NP_872604), RASSF6 (NP_803876), and RASSF7 (NP_001137466.1) as analyzed by the SMART computer program (http://smart.embl-heidelberg.de/). C1, protein kinase C conserved domains; RA, Ras association domains; SARAH, Salvador/RASSF/Hippo domains. Coiled-coil domains are also shown, as well as the number of amino acids in each protein. (**b**) Comparison of the amino acid sequences of the RA domains in RASSF1–6 and RASSF7. Alignments were generated using the ClustalW algorithm (Thompson *et al.*, 1994) and manually edited to maintain optimal overlapping. Identical and similar amino acids are boxed in black and gray, respectively. Asterisks indicate residues that are conserved in RASSF1–6 but not in RASSF7.

Supplementary Reference

1. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994 Nov 11; **22** (22)**:** 4673-4680.