eMethods

Structural modeling of VRK1-D263G variant

The 3D structure of the human Vaccinia-Related Kinase 1 (VRK1) wild-type protein was obtained from the Protein Data Bank (PDB id: 2LAV)[1]. Model for VRK1-D263G variant was generated using the wild-type structure as template. Models were built using the SWISS-MODEL server (http://swissmodel.expasy.org) and their structural quality were within the range of those accepted for homology-based structure (Anolea/Gromos/QMEAN4) [2]. Prior to molecular dynamics (MD) procedures, 3D structures were energy minimized using the GROMOS 43B1 force field implemented in DeepView (http://spdbv.vital-it.ch/), using 500 steps of steepest descent minimization followed by 500 steps of conjugate-gradient minimization.

Structures for wild-type and variant proteins were subjected to 200 ns of unrestrained Molecular Dynamics (MD) simulation using the AMBER18 molecular dynamics package (http://ambermd.org/; University of California-San Francisco, CA), essentially as previously described [3]. In brief, 3D models were first solvated with a periodic octahedral pre-equilibrated solvent box using the LEaP module of AMBER, with 12 Å as the shortest distance between any atom in the protein subdomain and the periodic box boundaries. Free MD simulation was performed using the PMEMD program of AMBER18 and the ff14SB force field (http://ambermd.org/), applying the SHAKE algorithm, a time step of 2 femtoseconds (fs) and a non-bonded cut-off of 12Å. Systems were initially relaxed over 10,000 steps of energy minimization, using 1,000 steps of steepest descent minimization followed by 9,000 steps of conjugate-gradient minimization. Simulations were then started with a 20 picoseconds (ps) heating phase, raising the temperature from 0 to 300 K in 10 temperature change steps, after each of which velocities were reassigned. During minimization and heating, the Cα trace dihedrals were restrained with a force constant of 500 kcal mol^{-1} rad^{-2} and gradually released in an equilibration phase in which the force constant was progressively reduced to 0 over 200 ps. After the equilibration phase, 200 ns of unrestricted MD simulation were obtained for the structures. MD trajectories were analyzed using VMD software [4]. Figures were generated using the Pymol Molecular Graphics System (https://pymol.org/; Schrödinger, LLC, Portland, OR).

Plasmids and mutagenesis

Human VRK1 was expressed from mammalian expression vector, pCEFL-HA-VRK1 [5], and bacterial expression pGEX-4T-VRK1 as previously describeb [5-8]. Human VRK1, kinase-dead VRK1-K179E and the VRK1-D263G variant were purified from constructs made in the pGEX4T-GST-VRK1 plasmid, and expressed in *E.coli* BL21 competent cells. The following plasmids were used to express the substrates: pGST4T-53BP1 (1-346) [9, 10]; GST-p53(1-85)[11] [6, 12], and pGEX4T-GST-Coilin(1-109) [13]. All plasmids were expressed in BL21 *E.coli* to purify the fusion protein used as substrate in kinase assays as previously reported [8, 13]. Human H3 and H2AX are purified recombinant proteins (Merck-Millipore).

Kinase assays

The kinase assays were performed as previously described [5, 8, 14]. Briefly, In vitro kinase assays with [^{32}- P]-γATP were performed with GST-VRK1 wild-type and variants [5, 7, 13]. Assays with the following substrates were previously published: p53 [12, 15], histone H3 [7, 16], 53BP1 [10], ATF2 [17], H2AX [16] and GST-coilin [13].

To perform the kinase assay, as substrates we used 2 µg of the VRK1 variant, 2 µg of the specific substrate (GST-53BP1(1-346), GST-Coilin(160-214), GST-p53(1-84), ATF2(1-109), and human recombinant histones H3 and H2AX, in a specific casein-kinase buffer (20mM Tris-HCl pH 7.5, 5mM $MgCl_2$, 0.5mM DTT and 150mM KCl), 5µM ATP and 5µCi (0.1µM) radiolabelled [ɣ-^{32}P]ATP in a final volume of 40µl. The reaction mix was incubated during 45 min at 30ºC [8]. The specific phosphorylation on the Thr3 of H3 and Thr18 of p53 were detected with a rabbit polyclonal antibody (Upstate-Millipore) [7, 16]. In radioactive assays, film exposure was in the lineal response range for all assays.

Electrophoresis, antibodies and immunoblots

Proteins were separated in SDS-PAGE gels in running buffer (25mM Tris-HCl, ph 8.0, 200mM glycine, 1.7mM SDS), and transferred to a PVDF membrane (Immobilon-FL, Millipore) using another specific buffer (25mM Tris-HCl, pH 8.0, 19.2 mM glycine, 15% methanol) as previously described [7, 13, 18, 19]. The primary and secondary antibodies are listed in Supplementary Table S1. The secondary antibodies were incubated for an hour and the fluorescence was detected with LI-COR Odyssey

Infrared Imaging System, or with ECL Western Blotting Detection Reagent (Sigma-Aldrich) depending on whether the secondary antibodies were conjugated with peroxidase.

Cell lines, transfections, cell lysates and protein stability

For the study of Cajal bodies formation, the validated HeLa (ATCC-CCL2) cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) and transfected using Lipofectin [7, 13, 18]. Cell extracts were prepared by using a mild lysis buffer (50mM Tris-HCl, pH 8.0, 150mM NaCl, 1% Triton X-100 and 1mM EDTA) supplemented with protease inhibitors (1mM PMSF, 10 µg/mL aprotinin and 10 µg/mL leupeptin) and phosphatase inhibitors (1mM sodium orthovanadate, 1mM NaF) [7, 8, 13].

In protein stability assays, cells were transfected with the corresponding plasmid and 72 hours later cycloheximide was added at 50µg/ml, samples were taken at the indicated times from cycloheximide addition, washed and lysed for analysis of protein level in immunoblots as previously use for all other VRK1 mutants [8]. Cells start to lose viability at approximately 36-48 hours [8].

Statistical analysis

Statistical analysis were performed using the IBM SPSS 28 statistics package. All assays were performed in the lineal response range and in identical conditions for all substrates [8]. Individual quantitative experiments were repeated three times, and statistical significance was analyzed using two-tailed T-test with Welch' correction [20].

Reagents

Recombinant human histones H3 and H2AX (Millipore, Merck). All other chemical were from Sigma-Merck (Darmstadt, Germany). Tissue culture media and reagents were from GIBCO-ThermoFisher Scientific (Waltham, MA).

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