

Targeting Hodgkin and Reed-Sternberg cells with an inhibitor of heat-shock protein 90: Molecular pathways of response and potential mechanisms of resistance

Priscilla Segges^a, Stephany Corrêa^b, Bárbara Du Rocher^{b,c}, Gabriela Vera-Lozada^a, Flavia Krsticevic^{a,d}, Debora Arce^{d,e}, Cinthya Sternberg^{f,g}, Eliana Abdelhay^b, Rocio Hassan^a

^aOncovirology Laboratory, Bone Marrow Transplantation Center (CEMO), Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; ^bLaboratório Célula-Tronco, CEMO, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; ^cLaboratório de Pesquisa sobre o Timo, FIOCRUZ, Rio de Janeiro, Brazil; ^dCIFASIS - Centro Internacional Franco Argentino de Ciencias de la Información y de Sistemas, Rosario, Argentina; ^eIICAR-CONICET Instituto de Ciencias Agrarias de Rosario, Rosario, Argentina; ^fLaboratório de Pesquisa Translacional, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; ^gcurrent addres: Programa de Pós-Graduação em Anatomia Patológica, Faculdade de Medicina da Universidade Federal do Rio de Janeiro, Brazil.

CORRESPONDENCE TO: Priscilla Segges.

Instituto Nacional de Câncer, Praça Cruz Vermelha, n° 23, 6° andar, ala C, CEMO, Laboratório de Oncovirologia, CEP: 20230130, Rio de Janeiro, RJ, Brazil. Telephone: 5521-3207-1874 Fax: 5521-3207-2121 Email: psegges@inca.gov.br

Supplementary File 1

Reagents

All reagents and solvents employed were commercially available and used as recommended by the manufacturers. RPMI 1640, streptomycin/penicillin for cell culture, 0.4% trypan blue vital stain, propidium iodide (PI), RNase and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (St. Louis, MO). 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1), protease inhibitor cocktail and ECL Plus® for western blot were purchased from Roche (Mannheim, Germany). Fetal bovine serum (FBS) was obtained from Gibco Life Technologies (Grand Island, NY). Annexin V-FITC Apoptosis Detection Kit and Caspase-Glo® 3/7 Assay Kit and ReliaPrep™ were purchased from BD Biosciences (Franklin Lakes, NJ) and Promega Corporation (Madison WI), respectively.

Antibodies against phospho-p44/42 (p-ERK1/2, D13.14.4E), p44/42 (ERK1/2), RAS (27H5), HSP70, HSF-1, HSP27 (G31), and anti-rabbit or mouse IgG HRP-linked were purchased from Cell Signaling Technology, Inc (Beverly, MA). Anti-actin and phospho-c-Fos antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, Calif) and Abcam (Cambridge, MA), respectively.

Celastrol with purity greater than 98% was purchased from Cayman Chemical (Ann Arbor, MI, USA).