

A combined computational and functional approach identifies IGF2BP2 as a driver of chemoresistance in a wide array of pre-clinical models of colorectal cancer

Sandra Kendzia¹, Susanne Franke¹, Tarek Kröhler², Nicole Golob-Schwarzl^{3,4,5}, Caroline Schweiger^{3,6}, Anna M. Toeglhofer³, Christina Skofler^{3,4}, Stefan Uranitsch⁷, Amin El-Heliebi^{4,8,9}, Julia Fuchs^{8,10}, Andreas Punschart¹¹, Philipp Stiegler¹¹, Marlen Keil¹², Jens Hoffmann¹², David Henderson¹³, Hans Lehrach¹⁴, Marie-Laure Yaspo¹⁴, Christoph Reinhard^{15,16}, Reinhold Schäfer⁶, Ulrich Keilholz⁶, Christian Regenbrecht^{16,17}, Rudolf Schicho¹⁸, Peter Fickert¹⁹, Sigurd F. Lax²⁰, Frank Erdmann¹, Marcel H. Schulz²¹, Alexandra K. Kiemer², Johannes Haybaeck^{3,22}, Sonja M. Kessler^{*1,2,3,23}

¹ Institute of Pharmacy, Experimental Pharmacology for Natural Sciences, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany

² Department of Pharmacy, Pharmaceutical Biology, Saarland University, Saarbrücken, Germany

³ Diagnostic & Research Center for Molecular Biomedicine, Institute of Pathology, Medical University of Graz, Graz, Austria

⁴ Center for Biomarker Research in Medicine (CBmed), Graz, Austria

⁵ Department of Dermatology and Venereology, Medical University of Graz, Austria

⁶ Charité Comprehensive Cancer Center, Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany

⁷ Department of Surgery, Hospital Brothers of Charity Graz, Austria

⁸ Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Medical University of Graz, Austria

⁹ BioTechMed-Graz, Graz, Austria

¹⁰ Division of Medical Physics and Biophysics, Medical University Graz, Austria

¹¹ Department of Surgery, Medical University of Graz, Austria

¹² Experimental Pharmacology & Oncology Berlin GmbH-Berlin-Buch, Germany

¹³ Bayer AG, Berlin, Germany

¹⁴ Max Planck Institute for Molecular Genetics, Berlin, Germany

¹⁵ Eli Lilly & Company, Indianapolis, USA

¹⁶ CELLphenomics GmbH, Germany

¹⁷ Institute for Pathology, University Hospital Göttingen, Göttingen, Germany

¹⁸ Division of Pharmacology, Medical University of Graz, Austria

¹⁹ Division of Gastroenterology and Hepatology, Medical University of Graz, Austria

²⁰ Department of Pathology, Hospital Graz South-West and School of Medicine, Johannes Kepler University Linz, Austria

²¹ Institute for Cardiovascular Regeneration, Goethe-University Hospital, 60590 Frankfurt, Germany

²² Institute of Pathology, Neuropathology and Molecular Pathology, Medical University of Innsbruck, Innsbruck, Austria

²³ Halle Research Centre for Drug Therapy (HRCDT)

Supplementary method

Western blot

Cells were lysed in lysis buffer (50 mM Tris-HCl, 1% (m/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.004% (m/v) bromophenol blue) supplemented with 1 mM sodiumorthovanadate, 1 mM PMSF and a protease inhibitor mixture (Complete®, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was done using the Bio-Rad Mini PROTEAN system (Bio-Rad, München, Germany). The Mini-Transblot cell (Bio-Rad, München, Germany) system was employed to transfer separated protein samples onto a polyvinylidene fluoride (PVDF) membrane (Immobilon-FL, Millipore, Schwalbach am Taunus, Germany). Membrane was blocked using Rockland Blocking Buffer (#MB-070, Rockland Immunochemicals). Antibodies used were specific for IMP2/p62¹ and b-actin (#D6A8, Cell Signaling). Both primary antibodies were diluted 1:1000 in Rockland Blocking Buffer and incubated for 1h at room temperature. Secondary HRP-conjugated antibody (Dianova) was diluted 1:5000 in 5% milk powder in TBS-T and incubated for 1 h inkubation at room temperature. For detection ECL Prime Western Blotting Detection Reagent (GE Healthcare Amersham) was used according to manufacturer's guidelines. Detection was performed with the Octoplus QPLEX Analyzer (nhDiagnostics, Halle, Germany).

1 Lu M, Nakamura RM, Dent ED, et al. Aberrant Expression of Fetal RNA-Binding Protein p62 in Liver Cancer and Liver Cirrhosis. *Am J Pathol* 2001;159(3):945-53.