NanoPro™ Assay: Survivin

SUMMARY:

Primary Antibody: Anti-Survivin (Cell Signaling Technology, cat# cs-2806)
Detection Antibody: Anti-Rabbit HRP (GE Healthcare, Product Code NA934)

Survivin, an inhibitor of apoptosis protein (IAP) is a pro-survival molecule that is increased in nearly every human tumor studied. In head and neck squamous cell carcinoma, Survivin levels are significantly greater than in normal upper aerodigestive mucosa. High Survivin levels in these tissues correlate with a higher probability of nodal metastasis and loco-regional recurrence, but may also indicate higher radiosensitivity. Survivin includes multiple sites for post-translational modification, including 4-5 major phosphorylation sites.

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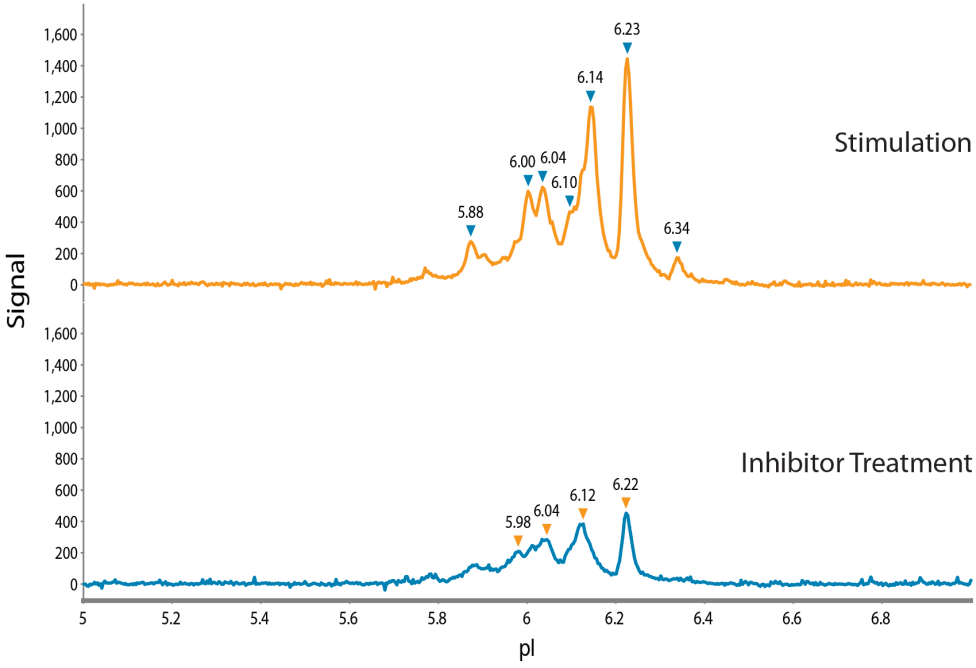
RESULTS:



DETECTION OF SURVIVIN IN OSC19 HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINE

OSC19 cells were treated with either 150 µM OSI906 for 2 hours followed by stimulation with des [1-3] IGF for 15 minutes (upper panel) or treated with 100 µM of the Hif-1A inhibitor YC-1 for 24 hours (lower panel). Impact on Survivin expression in response to stimulation or inhibitor treatment correlated with band intensities detected by Western blot (data not shown).

PROTOCOL:

CELL PREPARATION	
Cell culture	OSC19 human tongue squamous cell carcinoma cells were obtained from the laboratory of Jeffrey N Myers, MD, PhD (M. D. Anderson). Cells were cultured in DMEM/F12 (Gibco, cat# 11330) containing 4% FBS (Gibco, cat# 16000), 1x Penicillin/Streptomycin/Glutamine (JRS Scientific, cat# 20020) in 6 cm dishes. Cells were split 1:5 every 3 days using 0.25% Trypsin (Cellgro, cat# 25-053-CI) at 37 °C for 3-5 minutes. Data shown from cells at passage 5.
Treatment	12 hours before treatment with OSI906, media was aspirated and replaced with DMEM/F12 containing 0.5% FBS. Cells were exposed to 150 µM OSI906 (dissolved in DMSO) for 2 hours before stimulation with 10 nM des [1-3] IGF for 15 minutes, (des [1-3] IGF dissolved in 10 mM HCL and diluted in 0.5% BSA). Inhibitor treated cells were exposed to 100 µM YC-1 in DMSO for 24 hours.
Lysis buffer	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510) and 1x Aqueous Inhibitor Mix (ProteinSimple, p/n 040-482).
Lysis details	Media was aspirated, and cells were washed once with 2 mL ice-cold PBS. 100 µL ice-cold Bicine/CHAPS lysis buffer, supplemented with protease and phosphatase inhibitors, was added to cells before scraping into a pre-chilled microfuge tube. Cells incubated for 30 minutes in cold room on rotary shaker. Lysates clarified in centrifuge (16,100 x g for 15 minutes at 4 °C). Supernatants aliquoted (5 µL) for storage at -80 °C.
Storage	–80 °C

ASSAY REAGENTS	
Protein concentration	0.2 mg/mL final in capillary by BCA assay
Sample Diluent	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764), 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510), 40 mM DTT
Ampholyte Premix	Premix G2 pH 5-8 (ProteinSimple, p/n 040-973)
pI Standards	pI Standard Ladder 3 (ProteinSimple, p/n 040-646)
Wash	Wash Solution (ProteinSimple, p/n 040-313)
Primary antibody	Anti-Survivin (Cell Signaling Technology, cat# cs-2806, 1:50) in Antibody Diluent (ProteinSimple, p/n 040-309)
Detection antibody	Anti-Rabbit HRP (GE Healthcare, Product Code NA934, 1:100) in Antibody Diluent
Anolyte	Phosphoric Acid, 10 mM (ProteinSimple, p/n 040-337)
Catholyte	Sodium Hydroxide, 100 mM (ProteinSimple, p/n 040-338)
Luminol/Peroxide xDR	Mixed 1:1 (ProteinSimple, p/n 040-652 and p/n 040-653)

NOTE: NanoPro XDR Assays are now available with optional reagents to provide optimized assay sensitivity, extended dynamic range and both low and high end signal enrichment. Additionally, Premix G2 provides enhanced protein resolution. For more information, visit proteinsimple.com, contact your ProteinSimple Field Applications Specialist or call Technical Support at (888) 607-9692.

ASSAY CONDITIONS	
System	NanoPro 100
Sample Loading Time	25 seconds (Premix G2)
Focus Conditions	21000 µW, 40 minutes (Premix G2)
Immobilization	80 seconds
Wash 1	2 x 150 seconds (default)
Primary antibody incubation	120 minutes
Wash 2	2 x 150 seconds (default)
Detection antibody incubation	60 minutes
Wash 3	2 x 150 seconds (default)
Chemiluminescence exposure	480 and 960 seconds

OUR FAVORITE ANTIBODIES

Anti-Survivin (Cell Signaling Technology, cat# cs-2806)

SAMPLE PREPARATION ADDENDUM

Sample preparation procedures are different depending on the premix used. The following updated instructions provide guidance on sample preparation for both Premix G1 and Premix G2.

PREMIX G1 PROCEDURE	
Step 1	Dilute lysate with sample diluent to 0.1 mg/mL.
Step 2	In a separate tube, mix Premix G1 and pI standards.
Step 3	Mix equal parts of diluted lysate prepared in Step 1 with Premix G1 + pI Standards prepared in Step 2 (1:1 ratio) to create final protein concentration of 0.05 mg/mL.

PREMIX G2 PROCEDURE	
Step 1	Dilute lysate with sample diluent to 0.2 mg/mL.
Step 2	In a separate tube, mix Premix G2 and pI standards.
Step 3	Mix 1 part diluted lysate prepared in Step 1 with 3 parts Premix G2 + pI Standards prepared in Step 2 (1:3 ratio) to create final protein concentration of 0.05 mg/mL.

NOTE: When working with Premix G2, thorough mixing and vortexing during sample preparation is required. Additional sample volume may be required, 12-20 µL per sample well is recommended. Centrifugation of the sample plate (3000 x g, 10 minutes) is required.

For further assistance, please contact your ProteinSimple Field Applications Specialist or Technical Support at (888) 607-9692.

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