Bio-Plex™ suspension array system tech note 5296

Simultaneous Detection of Multiple Phosphoprotein Targets From Human Tumor Tissues Using the Bio-Plex Suspension Array System

Qian Gao, Kris Simonyi, Claudia Suen, Judy Zhu-Shimoni, and Sophie Allauzen, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

Introduction

We studied three human cancer tissues with the Bio-Plex suspension array system and Bio-Plex phosphoprotein assays. The Bio-Plex suspension array system is based on Luminex xMAP technology (Spain 1998) and can detect multiple targets simultaneously (Figure 1). The Bio-Plex system has been successfully used to assay biological protein targets, such as cytokines (Prabhakar et al. 2002) and kinases. We have developed a multiplex panel of assays that can detect 18 phosphoproteins simultaneously from cultured cell lysates or tissue lysates (Gao 2003). Different phosphorylation levels were detected in 8 of 18 targets, and their signals (median fluorescence intensity or MFI) were compared with western blot results.

Methods

The tissue lysates used were PC3 for prostate cancer, Calu-3 for lung cancer, and HCT-116 for colorectal cancer (provided by Roche Diagnostics, Germany). Tissue lysates were prepared according to the instructions specified in the Bio-Plex cell lysis kit product insert. Multiplex phosphoprotein detection was performed using Bio-Plex phosphoprotein assays. Western blot analysis was done with routine procedures, and the blots were probed with corresponding phospho-specific antibodies. The protocols for tissue lysate preparation and assays are summarized in Figure 2.

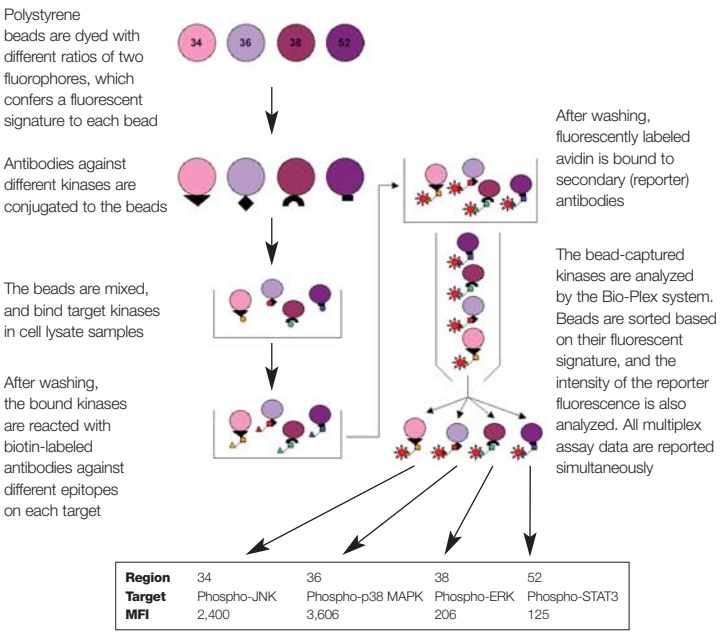

Fig. 1. xMAP technology.

Tissue Lysate Preparation	Assay Procedure
Wash tissue 1x and cut into 3 x 3 mm pieces	Prewet filter plate
	Add antibody-conjugated beads
Homogenize in cell lysis buffer	**wash**
	Incubate with cell lysate in assay buffer
Freeze and thaw 1x	**wash**
Sonicate briefly	Incubate with detection antibodies
	wash
Centrifuge to remove debris	Incubate with streptavidin-PE
Collect supernatant	**wash**
	Resuspend beads
Adjust protein concentration and run Bio-Plex phosphoprotein assay (10 µg/test)	Read on Bio-Plex suspension array system

Fig. 2. Protocols for tissue lysate preparation and assays.

BIO-RAD

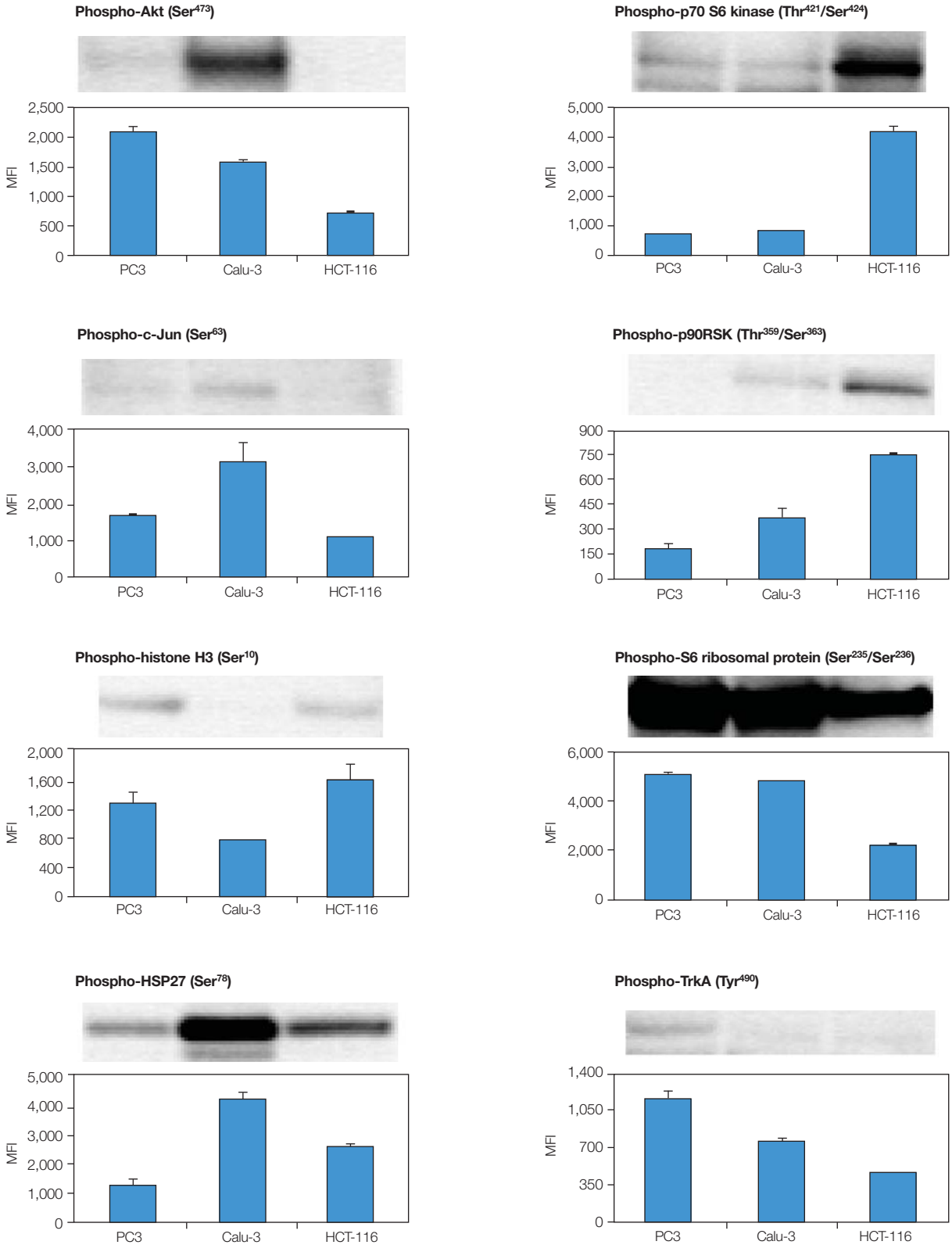

Fig. 3. Correlation of Bio-Plex phosphoprotein assays (lower panels) with western blots (upper panels) for the 8 targets showing increased phosphorylation levels. Note correlation of assay MFI values to band intensities.

Table 1. Target proteins and their phosphorylation sites.

Target	Sites	Target	Sites
Phospho-Akt	Ser^{473}	Phospho-NF-κB p65	Ser^{536}
Phospho-ATF-2	Thr^{71}	Phospho-p38 MAPK	Thr^{180}, Tyr^{182}
Phospho-c-Jun	Ser^{63}	Phospho-p53	Ser^{15}
Phospho-ERK1/2	Thr^{202}/Tyr^{204}, Thr^{185}/Tyr^{187}	Phospho-p70 S6 kinase	Thr^{421}, Ser^{424}
Phospho-GSK-3α/β	Ser^{21}/Ser^{9}	Phospho-p90RSK	Thr^{359}, Ser^{363}
Phospho-histone H3	Ser^{10}	Phospho-S6 ribosomal protein	Ser^{235}/Ser^{236}
Phospho-HSP27	Ser^{78}	Phospho-STAT2	Tyr^{690}
Phospho-IκB-α	Ser^{32}/Ser^{36}	Phospho-STAT3	Tyr^{705}
Phospho-JNK	Thr^{83}, Tyr^{85}	Phospho-TrkA	Tyr^{490}

Results and Discussion

The preliminary data obtained for the three human cancer tissue lysates studied were very promising. Among 18 tested phosphoprotein targets (Table 1), 8 showed increased phosphorylation levels. All positive results were compared with western blot analysis, and good correlation was found for 7 of 8 phosphoprotein targets (Figure 3). For phospho-Akt (Ser^{473}), the Bio-Plex assay detected a higher phosphorylation level in the human prostate cancer sample (PC3) than the lung cancer (Calu-3) and colorectal cancer (HCT-116) samples. However, the western blot results showed the highest phosphorylation level for lung cancer. This discrepancy in the cancer tissue samples is under investigation.

Conclusions

Applying Bio-Plex phosphoprotein assays to human cancer tissue lysates provided an efficient way to detect multiple phosphoprotein targets simultaneously with tissue lysates. More cancer tissue lysates need to be tested to reveal a better profile of multiple phosphoprotein status.

References

Gao Q, Simultaneous detection of eight phosphorylated and four total proteins using the Bio-Plex protein array system, Mol Biol Cell 14, 152a (2003)

Prabhakar U et al., Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP assay, J Immunol Methods 260, 207–218 (2002)

Spain M, Multiplexed testing for disease-marking synergies, IVD Technology 4, 34–38 (1998)

Information in this tech note was current as of the date of writing (2005) and not necessarily the date this version (Rev A, 2005) was published.

CST antibodies are developed and validated exclusively for Bio-Rad.

Bio-Rad
Laboratories, Inc.

Life Science
Group

Web site www.bio-rad.com **USA** 800 4BIORAD **Australia** 02 9914 2800 **Austria** 01 877 89 01 **Belgium** 09 385 55 11 **Brazil** 55 21 3237 9400
Canada 905 712 2771 **China** 86 21 6426 0808 **Czech Republic** 420 241 430 532 **Denmark** 44 52 10 00 **Finland** 09 804 22 00 **France** 01 47 95 69 65
Germany 089 318 84 0 **Greece** 30 210 777 4396 **Hong Kong** 852 2789 3300 **Hungary** 36 1 455 8800 **India** 91 124 4029300/5013478 **Israel** 03 963 6050
Italy 39 02 216091 **Japan** 03 5811 6270 **Korea** 82 2 3473 4460 **Mexico** 55 5200 05 20 **The Netherlands** 0318 540666 **New Zealand** 64 9415 2280
Norway 23 38 41 30 **Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700 **Russia** 7 095 721 14 04 **Singapore** 65 6415 3188 **South Africa** 27 0861 246 723
Spain 34 91 590 5200 **Sweden** 08 555 12700 **Switzerland** 061 717 95 55 **Taiwan** 886 2 2578 7189/2578 7241 **United Kingdom** 020 8328 2000

Bulletin 5296 US/EG Rev A 05-0444 1205 Sig 1205