


シンプルウェスタンの有用性

The usefulness of Simple Western

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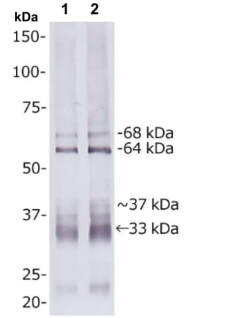
- Traditional western blot
- Simple Western
- Simple Western vs Traditional western blot
- An example of study using Simple Western

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Traditional western blot

➤ Western blot is a widely used analytical technique in molecular biology.

Western blot



Immunohistochemistry

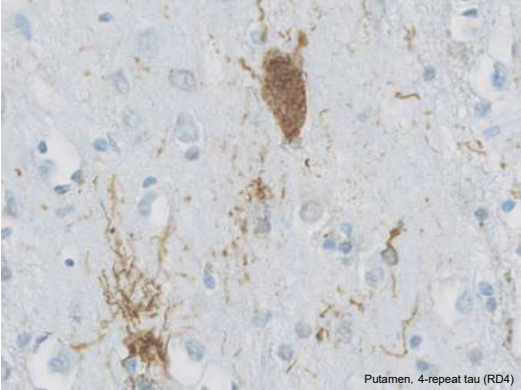


Fig. 4 Immunoblot analysis of sarkosyl-insoluble tau. In both lanes #1 (precentral gyrus) and #2 (putamen), hyperphosphorylated full-length 4-repeat tau bands (64 and 68 kDa) and C-terminal fragments (33 kDa) are visible.

Beck G, et al. *Neuropathology* 2023; 43(4): 326-332.

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Traditional western blot

➤ Western blot is the gold standard for identification and quantification of proteins.

	Southern blot	Northern blot	Western blot
Target molecule	DNA	RNA	Protein
Sample preparation	DNA extraction Enzymatic digestion	RNA isolation	Protein extraction
Separation	Electrophoresis	Electrophoresis	Electrophoresis
Membrane material	Nylon	Nylon	PVDF Nitrocellulose
Probe	Nucleic acid probe with sequence homologous to target	RNA, DNA, or oligodeoxynucleotide	Primary antibody
Probe label	Radiolabel, enzyme	Radiolabel, enzyme	enzyme

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Traditional western blot

➤ The prototype of Western blot was first described by Towbin H in 1979.

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 9, pp. 4350-4354, September 1979
Biochemistry

Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications

(ribosomal proteins/radiimmunoassay/fluorescent antibody assay/peroxidase-conjugated antibody/autoradiography)

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Communicated by V. Prelog, June 12, 1979

FIG. 1. Assembly for electrophoretic blotting procedure. 1, Electrodes of destainer; 2, elastic bands; 3, disposable pipette-tip tray; 4, nitrocellulose sheets; 5, polyacrylamide gel; 6, Scotch-Brite pads. Assembly parts are shown separated for visualization only.

Towbin H, et al. *Proc Natl Acad Sci U S A*. 1979; 76(9): 4350-4.

FIG. 4. Detection of E. coli ribosomal protein L7 and L12 by (A) horseradish peroxidase- and (B) fluorescein conjugated antibodies. Total ribosomal proteins from E. coli were separated and blotted as in Fig. 2. The anti-L7,L12 serum had a dilution of 100 times of 10% ribosomal serum as determined by turbidity formation (20). Incubation was for 3 hr at room temperature in goat antiserum diluted 1:10 in saline containing 1% bovine serum albumin and 10% rabbit carrier serum and then with conjugated anti-goat IgG. In each case the lower strip is a control with peroxidase antiserum. Electrophoresis was from left to right.

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Traditional western blot

➤ The method was named "Western blot" after Southern blot and Northern blot.

ANALYTICAL BIOCHEMISTRY 112, 195-203 (1981)

"Western Blotting": Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose and Radiographic Detection with Antibody and Radioiodinated Protein A

W. NEAL BURNETTE†

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104
Received May 20, 1980

A B

gP95/85⁹⁰⁹ — 97.4 Kd
P75⁹⁰⁹ — 66.3 Kd
Pr65⁹⁰⁹ — 43 Kd
— 30 Kd

distortion, and antigen denaturation. With due respect to Southern (1), the established tradition of "geographic" naming of transfer techniques ("Southern," "Northern") is continued; the method described in this manuscript is referred to as "Western" blotting.

FIG. 4. Western blot and antibody-IPA detection of intracellular MuLV-specific antigens. Lysates of AKR MuLV-infected E δ G2 mouse cells and Moloney MuLV-infected C3H mouse cells were subjected to SDS gel electrophoresis in a 10% polyacrylamide slab gel and the fractionated proteins subsequently transferred to nitrocellulose paper. The blot was then reacted with rabbit antiserum directed against the major internal structural protein (p30) of MuLV and the immune complexes illuminated with IPA followed by radioautography. Lane A contained lysate equivalent to 3×10^5 E δ G2 cells and lane B to 1×10^5 C3H cells. Exposure time of this radioautogram was 15 min.

Burnette WN. *Anal Biochem* 1981; 112(2): 195-203.

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Traditional western blot

➤ The basic principle of Western blot technique has remained virtually the same since developed.

Separation

Separate
proteins by gel electrophoresis.

Transfer

Transfer
proteins from gel to membrane.

Detection

Detect
the target protein by antigen-antibody reaction.

Visualization

Visualize
the detected protein as a band image.

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Traditional western blot

➤ Sample preparation

Wash Lysis

Centrifuge Collect supernatant

SDS 2ME Heat block

➤ SDS (sodium dodecyl sulfate) ... detergent, protein denature, negative charge

➤ 2ME (2-mercaptoethanol) ... protein reducing agent

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Traditional western blot

➤ Separation

➤ Negatively charged proteins run through polyacrylamide gel.
➤ Unfolded proteins are separated by molecular size.

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Traditional western blot

➤ Transfer

➤ Two methods are mainly used; wet transfers or semi-dry transfers
➤ Separated proteins in gel are transferred to blotting membrane (PVDF or Nitrocellulose).

Lück C, et al. Methods Mol Biol. 2021; 2261: 481-488.

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Traditional western blot

➤ Detection

➤ Target proteins are detected based on antigen-antibody reaction.
➤ Secondary antibodies recognize primary antibodies which bond to target proteins.

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Traditional western blot

➤ Visualization

➤ The main techniques for visualizing a western blot is chemiluminescence.
➤ The emitted light is measured with a cooled CCD camera.

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Simple Western

➤ Simple Western™ is a novel western system from ProteinSimple.

Simple Western™ Automated Western Blot Systems

Gel-Free, Blot-Free, Hands-Free Capillary Western Blot Analysis from ProteinSimple, a Bio-Techne Brand

Request Pricing

Compare Models | Specifications | Consumables



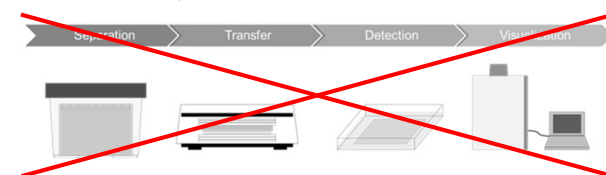
https://www.bio-technne.com/instruments/simple-western

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Simple Western



➤ Simple Western™ is fully automated.

Separation → Transfer → Detection → Visualization



↓







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Simple Western

➤ Simple Western™ is a gel-free, capillary-based western immunoassay.

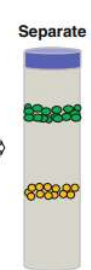
Load Matrix



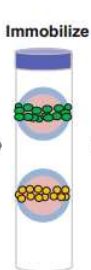
Load Sample




Separate



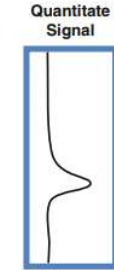
Immobilize



Immunoprobe



Quantitate Signal



■ Stacking Matrix

□ Separation Matrix

● High MW

● Low MW

⋈ Target Protein

⋈ Primary Ab

⋈ HRP-labeled Secondary Ab

Harris VM. *Methods Mol Biol.* 2015;1312:465-8.

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Simple Western

➤ Workflow of Simple Western™

Plate preparation → Separation → Transfer → Detection → Visualization

- Samples
- Blocking reagents
- Primary antibodies
- Secondary antibodies
- Chemiluminescence reagents

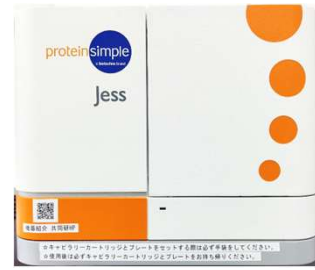
1 5 10 15 20

A B C D E F

Evaporation sensitive

Peel off immediately before placing in instrument

© ProteinSimple



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Simple Western

➤ If you want to start Simple Western™, minimum required equipment is only **Separation module**.

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Simple Western

➤ The only step you have to do manually is plate preparation among Simple Western™ procedure.

- Samples**
Samples used for traditional western blot is available
Need to adjust concentration
Mixed with some reagents in Separation module
- Blocking reagents**
Detection module Antibody Diluent 2 reagent
Other blocking reagents can be used
- Primary antibodies**
Antibodies to target proteins
Need higher concentration than in traditional western blot
- Secondary antibodies**
Detection module contains secondary antibodies
Other secondary antibodies can be used
Streptavidin-HRP for the ladder lane
- Chemiluminescence reagents**
Detection module contains Luminol-S and Peroxide
Other chemiluminescence reagents can be used

© ProteinSimple

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Simple Western

➤ Data generated by Simple Western™ apparatus are analyzed Compass software.

Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Width	S/N	Baseline	Channel
OVSARO	SMAR...	anti-rab...	2	1		522	195	950.6	14162.4	14.0	56.2	37.4	CHEMI	
OVATE	SMAR...	anti-rab...	3	1		468	159	415	1055.6	25.9	23.1	53.2	CHEMI	
OVATE	SMAR...	anti-rab...	3	2		521	197	376.6	5424.3	13.5	26.9	50.8	CHEMI	
NHIOVCAR-3	SMAR...	anti-rab...	4	1		458	100	72.3	1902.7	24.7	14.4	48.3	CHEMI	
NHIOVCAR-3	SMAR...	anti-rab...	4	2		468	141	188.1	5213.2	26.8	74.8	48.1	CHEMI	
NHIOVCAR-3	SMAR...	anti-rab...	4	3		520	196	804.3	10124.2	11.8	67.0	48.1	CHEMI	
KURAMOCHI	SMAR...	anti-rab...	5	1		420	64	70.9	1646.6	21.7	20.0	35.7	CHEMI	

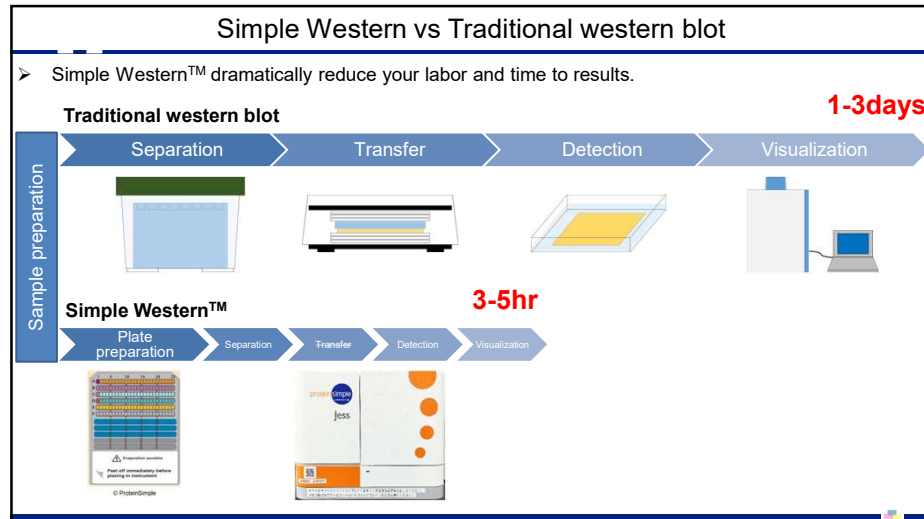
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Simple Western vs Traditional western blot

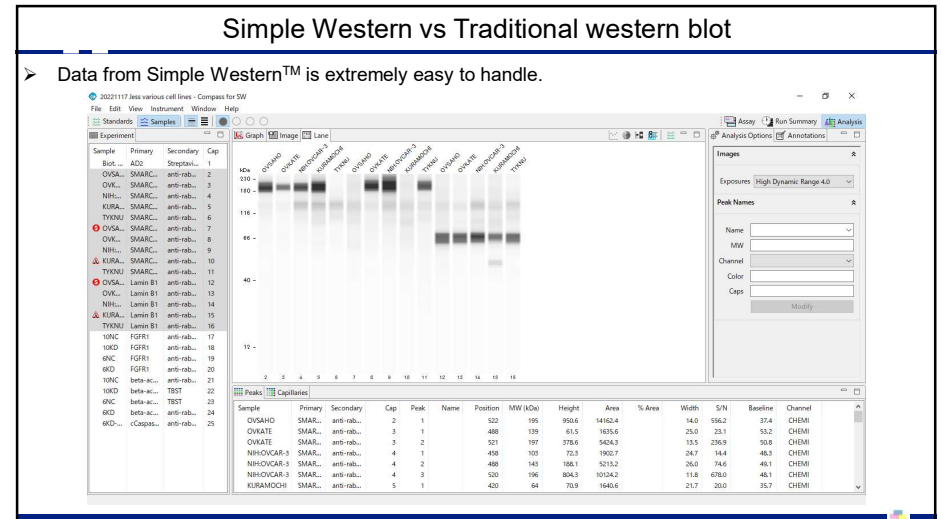
➤ Simple Western™ has a lot of advantages compared to traditional western blot.

	Simple Western	Traditional western blot
Labor	Fully automated	Many manual steps
Time	3-5hr	1-3days
Sample	3μL	10-20μL
Reproducibility	○	?
Visualization	⊙	△
Quantification	⊙	△

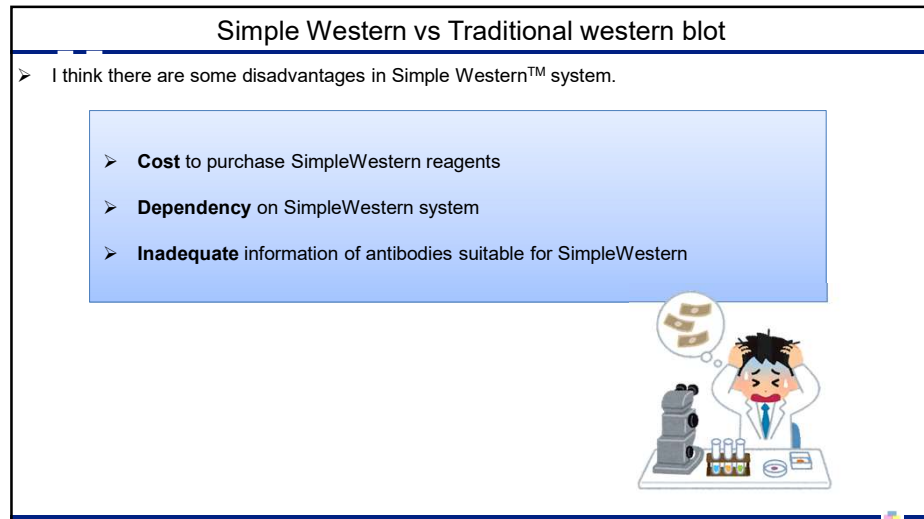
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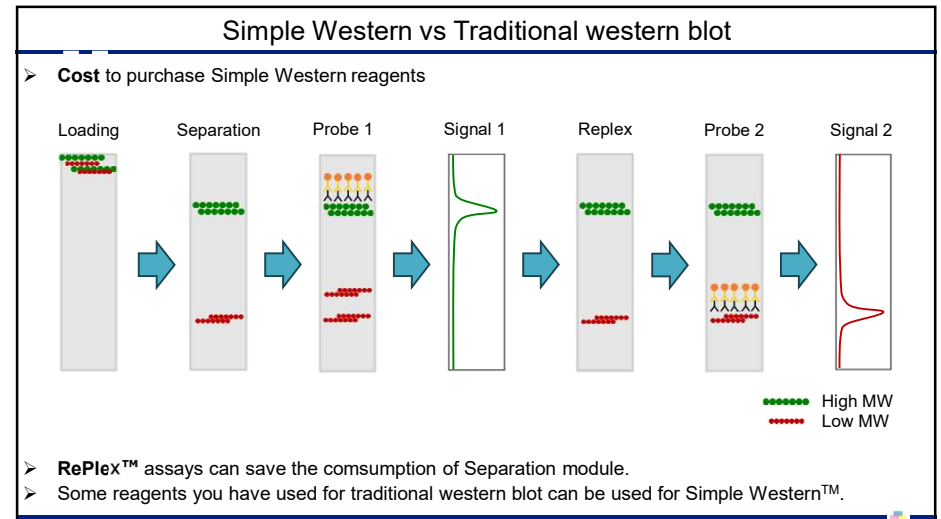
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Simple Western vs Traditional western blot

➤ **Dependency on SimpleWestern system**

Fully booked

Troubles

- If you only learn Simple Western™, you become unable to research without it.
- Two different western blot assays have a common basic principle.
- I recommend to learn traditional western blot before you use Simple Western™.

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Simple Western vs Traditional western blot

➤ **Inadequate information of antibodies suitable for SimpleWestern**

Simple Western Validated Antibodies

Protein Target	Antibody Type	Vendor / Cat #	Protein Isoform	Citations & Supporting Data	Host Species	Sample	Separation	Dilution	Kits on Simple Western
α-actinin	Primary	Santa Cruz Biotechnology sc-119245	Unmodified	10 Citations (1)	Mouse	NCI H1975	Size	Not Stated	Not Stated
α-Cardiac Actin	Primary	R&D Systems MAB356 CT	alpha-Cardiac Actin	15 Citations	Mouse, mouse, and rat heart tissue	Human, mouse, and rat heart tissue	Size	2 µg/ml	50

➤ Research papers using Simple Western™ is increasing.

➤ Manufacturers' validated antibody databases are being improved.

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Journal of Pathology / Pathol 2023
Published online 17 March 2023 in Wiley Online Library (wileyonlinelibrary.com) doi: 10.1002/path.5454

ORIGINAL ARTICLE

Ovarian high-grade serous carcinoma cells with low SMARCA4 expression and high SMARCA2 expression contribute to platinum resistance

Kanae Kido¹, Satoshi Nojima^{1*}, Daizuke Motooka^{1,2,3,4}, Yusuke Nomura¹, Masaharu Kohara¹, Kazuki Sato¹, Kenji Chahima¹, Shinichiro Tahara¹, Masako Kurashige¹, Daisuke Umeda¹, Tsuyoshi Takashima¹, Hiroki Kiyokawa¹, Koto Ukon¹, Takahiro Matsui¹, Daisuke Okuzaki^{1,5,6} and Eichi Mori^{1,4*}

Alteration of chromatin architecture

Chemoresistance

HGSC tissue

A4^{hi}/A2^{lo}
A4^{lo}/A2^{hi}
A4^{hi}/A2^{hi}
A4^{lo}/A2^{lo}

➤ The first report using Simple Western™ (Jess™) of Center for Medical Research and Education, Graduate School of Medicine, Osaka University.

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Signal intensity (A4)

Signal intensity (A2)

Cell number

Cell number

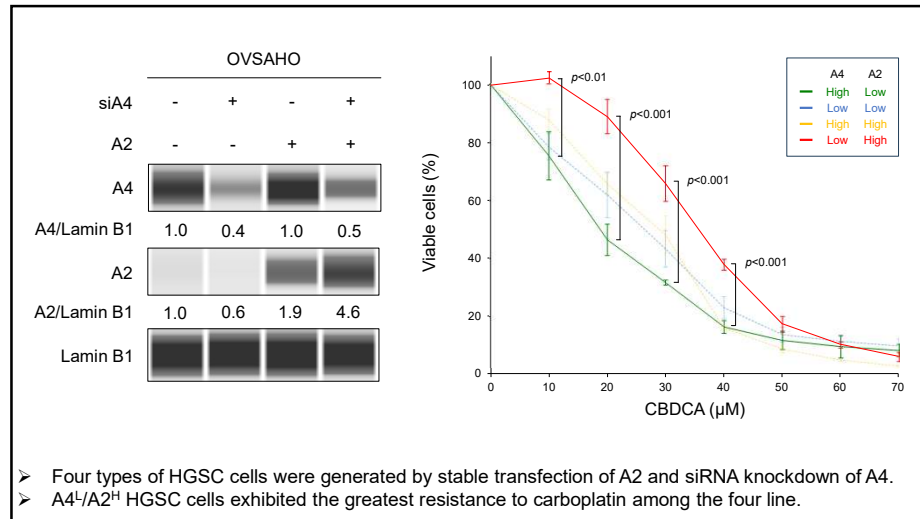
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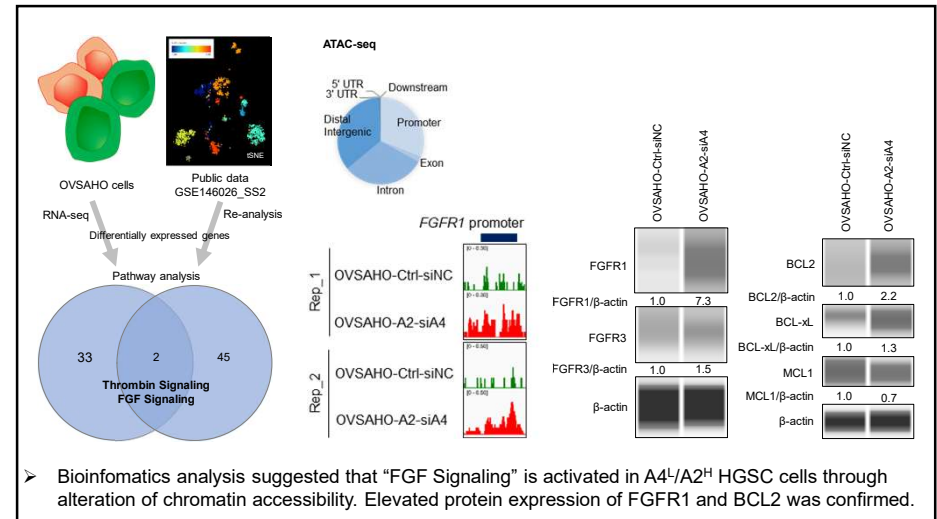
S: platinum sensitive, R: platinum resistant

➤ Fluorescent multiplex immunohistochemical analysis of ovarian HGSC tissue revealed that A4^{lo}/A2^{hi} cancer cell subpopulation was significantly increased in platinum-resistant cases.

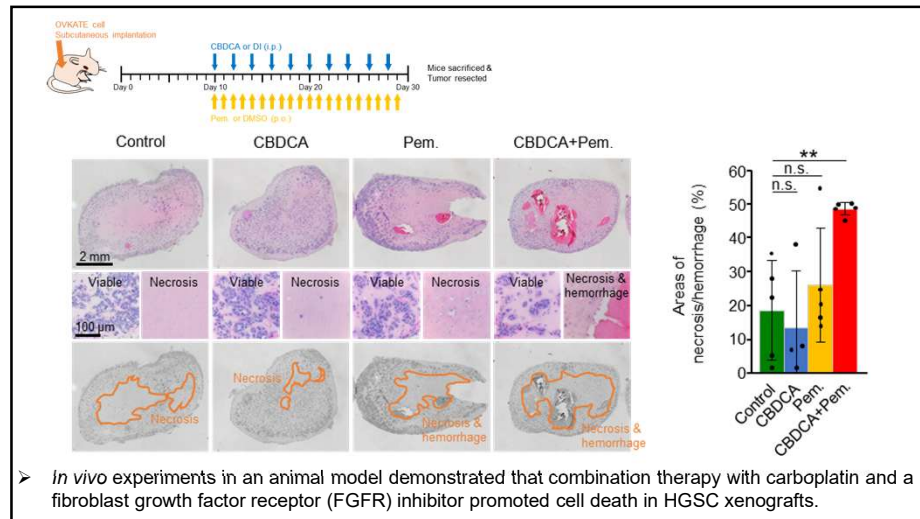
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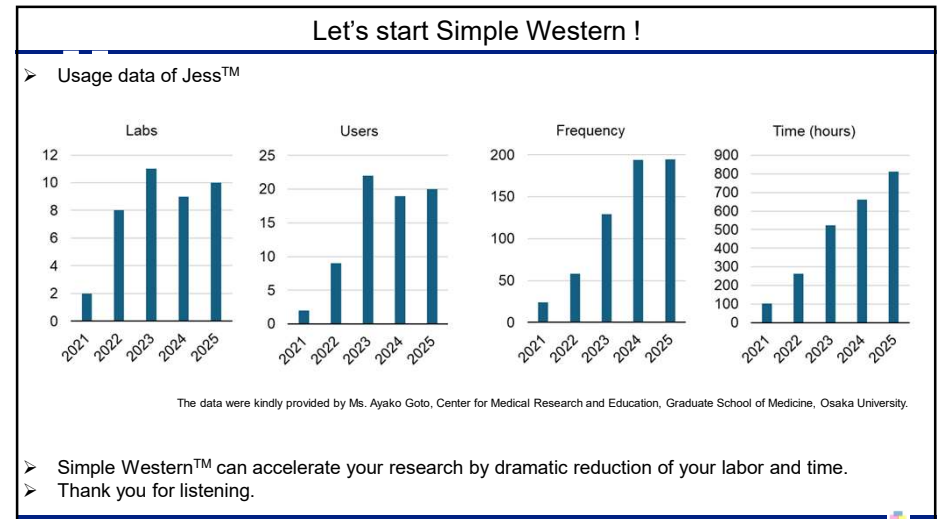
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