A comparison of protein expression in nonmalignant and malignant cells: *A novel protein with PCNA homology*

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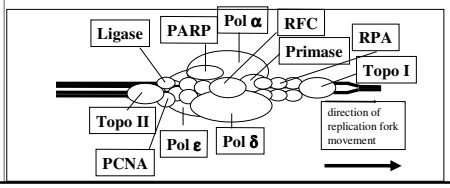
Genome changes in cancer

- Exhibit a high rate of DNA synthesis
- Extensive DNA damage, chromosome anomalies
- Altered DNA replication /repair
- Screen DNA replication proteins to find altered proteins specific for cancer (ovarian)



Proliferating Cell Nuclear Antigen (PCNA) PCNA and cancer

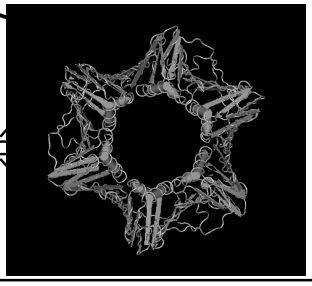
- PCNA expression is greater in proliferating cells and in cancer cells — He et al. 1994
- PCNA expression predictive of degree of malignancy — Schonborn et al. 1995
- Altered form of PCNA observed in breast cancer — Bechtel et al. 1998

Are there cancer specific PCNA isoforms?

Develop a novel antibody to PCNA

Strategy:
Target the part of PCNA that binds enzymes involved in DNA replication

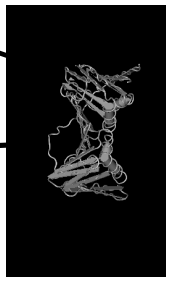
The B1 antibody



The B1 antibody is different because It binds to a part of PCNA that other PCNA antibodies do not bind to

Many commercial antibodies bind here, ex: PC-10 antibody

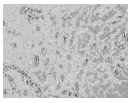
The B1 antibody binds here



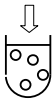
Comparison of cancer cell lines And normal cells

Methods:

Grow cell types in tissue culture



Prepare proteins from cells in a solution form (lysate)

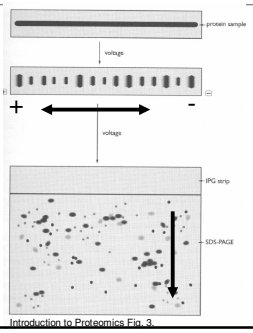


Separate proteins from each other using a two dimensional gel system (2D gels)

2 D gel electrophoresis

Isoelectric focussing

Separate by protein charge

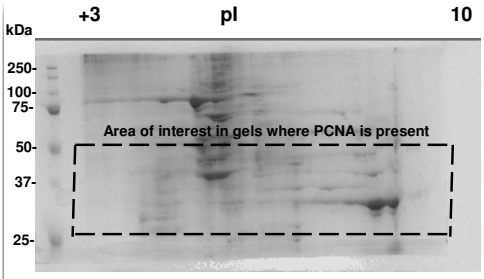


SDS PAGE gel

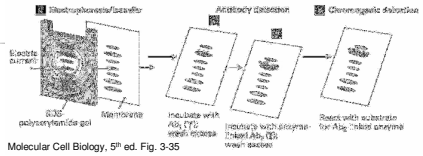
Separate by protein size

Introduction to Proteomics Fig. 3.

2D gel Ovarian epithelial cell proteins

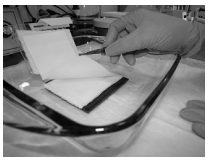


Western Blotting with B1 antibody

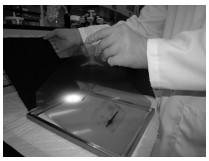


Molecular Cell Biology, 5th ed. Fig. 3-35

Transfer from gel to membrane

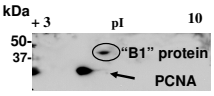


Capture of antibody reaction on film

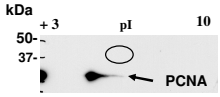


The B1 PCNA antibody detects a novel protein in normal epithelial cells

B1 antibody

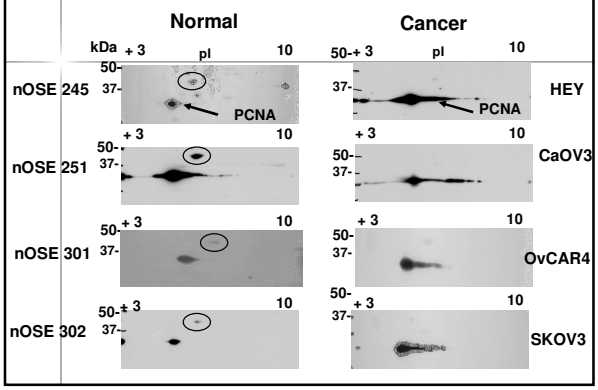


PC-10 antibody



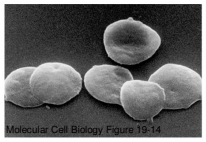
- The novel protein shares homology with PCNA based on detection by the B1 antibody
- The B1 protein is different from PCNA since other PCNA antibodies do not bind to it

B1 expression in ovarian cells

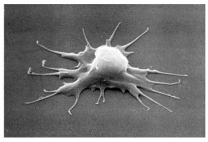


The B1 protein is characteristic of normal cells, but is absent or reduced in cancer cells.

Normal + B1 protein



Cancer – B1 protein



Molecular Cell Biology Figure 19-14

Is the B1 protein a tumor suppressor? Does B1 protein help control genome maintenance in normal cells?

What is the B1 protein ?

- Isolate and purify the B1 protein
- Analysis by mass spectrometry

Current results

The B1 protein could be a form of actin

	124		133
PCNA	EQ L GIPEQEY		
	. : . : .: : :		
β-Actin	QQMWISKQEY		
	353		362

The B1 binding region in PCNA is similar to a region in β- actin

Cytoplasmic actin in the nucleus

Actin and gene transcription
Cell 39: 111(1984), J. Cell Biol. 153: 229 (2001), Nature Cell Biol. 6: 1094 (2004)

Actin linked to chromatin remodeling
Annu. Rev. Biochem. 71: 755 (2002), Genes Dev. 19: 1871 (2005)

Actin linked to cell transformation
Cell 79:119 (1994), Mol. Cell Biol. 16:1576 (1996)

Acknowledgements

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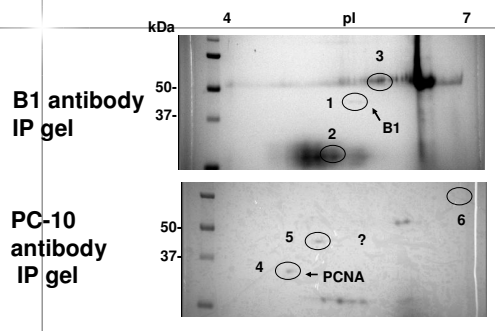
Immunoprecipitation protocol

- Prepare whole cell lysate from PA-1 cell line
- Pre-clear with protein A
- Immunoprecipitation (IP) of lysate with rabbit control IgG-AC (agarose conjugate)
- IP of lysate with PC-10-AC antibody to remove PCNA
- IP with B1 antibody and protein A

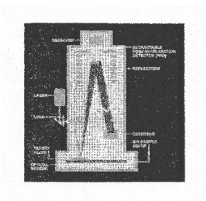
Immunoprecipitation products



Sample preparation for mass spectrometry

- Excision of sample spots, in-gel trypsin digest (2 and 4 hr), elution of peptides
- Co-crystallization of peptides with matrix (α-cyano-4-hydroxycinnamic acid)
- Collect peptide fingerprint by MALDI-TOF
- Database searching for finger print match

Waters Micromass MALDI micro MX instrument



Laser pulses of 1200 V, 10 shots per spectrum
Collected 20-50 spectra per sample
Peptides from 900 – 3000 Da detected,
Proteinlynx software processes spectra

MALDI-TOF spectra

B1 spot

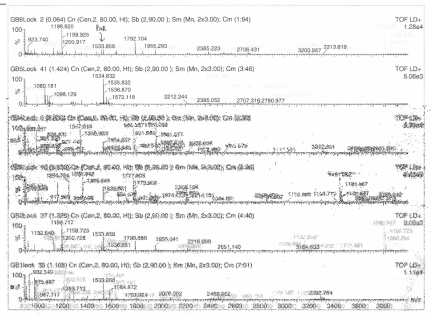
Blank

Heavy IgG

Light IgG

45kD spot

PCNA



Peptide fingerprint analysis

Analysis programs

MS-Fit	U.C.S.F., San Francisco, CA
Mascot	Matrix Science Ltd., London, U.K.
Profound	Rockefeller University, New York, NY

Databases searched:

NCBInr	Profound, Ms-Fit, Mascot
SwissProt	MS-Fit, Mascot
MSDB	Mascot

Search criteria

Based on monoisotopic peptide masses

Singly charged ions, m/z = MH+

Mass tolerance +/- 1 Da

Residue modifications: cysteine alkylation

Intact protein mass range and pI range available in MS-Fit and Profound

Search Results

Spot 4 (PCNA)

	Mascot		MS-Fit		ProFound	
	Protein	Score	Protein	Score	Protein	Score
NCBInr	PCNA	156	PCNA	$1.26xe^{+7}$	PCNA	$1.2x10^{-4}$
Swiss Prot	PCNA	160	PCNA	$2.01xe^{+8}$		
MSDB	PCNA	160				

Search results

Spot 1 (B1 protein)

	Mascot		MS-Fit		ProFound	
	Protein	Score	Protein	Score	Protein	Score
NCBInr	β-actin	105	β-actin	$6.04e^{+8}$	β-actin profilin complex	$3.3x10^{-4}$
Swiss Prot	β-actin	101	β-actin	$2.56e^{+9}$		
MSDB	β-actin	107				

Homologous domain in PCNA and actin

```
             124             133
PCNA         EQ L GIPEQEY
              . : .   :  . : : :
Actin        QQMWISKQEY
             353             362
```

50% identity in 10 aa overlap

- Contains B1 antibody antigenic sequence
- Represents section from interdomain connector loop in PCNA

Actin isoforms

ACTB β-actin, cytoplasmic 1
ACTG γ-actin, cytoplasmic 2
} Non-muscle

ACTH γ-enteric
ACTA α-vascular
} Smooth muscle

ACTC α-cardiac muscle
ACTS α-skeletal muscle
} Sarcomeric

Homology of actin isoforms

```
ACTB      MDDDIAALVVDNGSGMCKAFAGDDA.......
ACTG      MEEEIAALV I DNGSGMCKAFAGDDA.......
ACTH      MCEEETTALVCDNGSGLCKAFAGDDA.......
ACTA      MCEEEDSTALVCDNGSGLCKAFAGDDA.......
ACTC      MCDDEETTALVCDNGSGLCKAFAGDDA.......
ACTS      MCDEDETTALVCDNGSGLCKAFAGDDA.......

ACTB ..........STF[QQMWISKQEY]DESGPSIVHRKCF
ACTG ......... STF[QQMWISKQEY]DESGPSIVHRKCF
ACTA ......... STF[QQMWISKPEY]DEAGPSIVHRKCF
ACTH ......... STF[QQMWISKQEY]DEAGPSIVHRKCF
ACTC ......... STF[QQMWISKQEY]DEAGPSIVHRKCF
ACTS ......... STF[QQMWITKQEY]DEAGPSIVHRKCF
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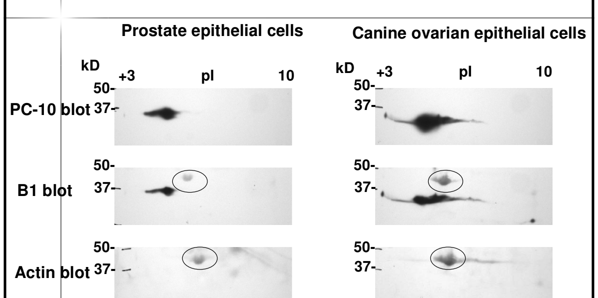
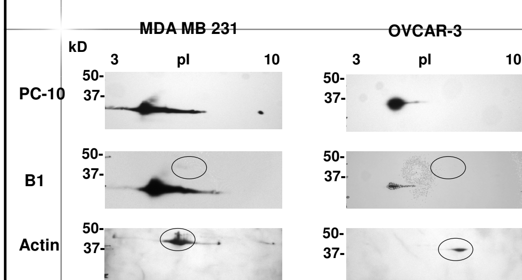
Comparison of B1 and Actin blots in Non-malignant primary epithelial cells



Comparison of B1 and Actin blots in Malignant ovarian epithelial cell lines



Conclusions

- The B1 PCNA antibody detects a protein different from PCNA in primary, normal cells and nonmalignant tissue.
- The B1 protein could be an isoform of actin.
- Reduced expression of the B1 protein may be associated with malignancy in epithelial cells.

Future Directions

- Determine if the B1 protein corresponds to an isoform of actin or an actin-related protein
- Screen other tissue and tumor types for the presence/absence of the B1 protein
- If the B1 protein is an isoform of actin, why is it not detected by the B1 antibody in all cell types?
- Is the B1 protein a component of nuclear protein comlexes?
- Is expression of the B1 protein associated with specific cell compartments? At specific stages of the cell cycle?

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