THE ROLE OF UV IN MELANOMA INDUCTION IN XERODERMA PIGMENTOSUM PATIENTS

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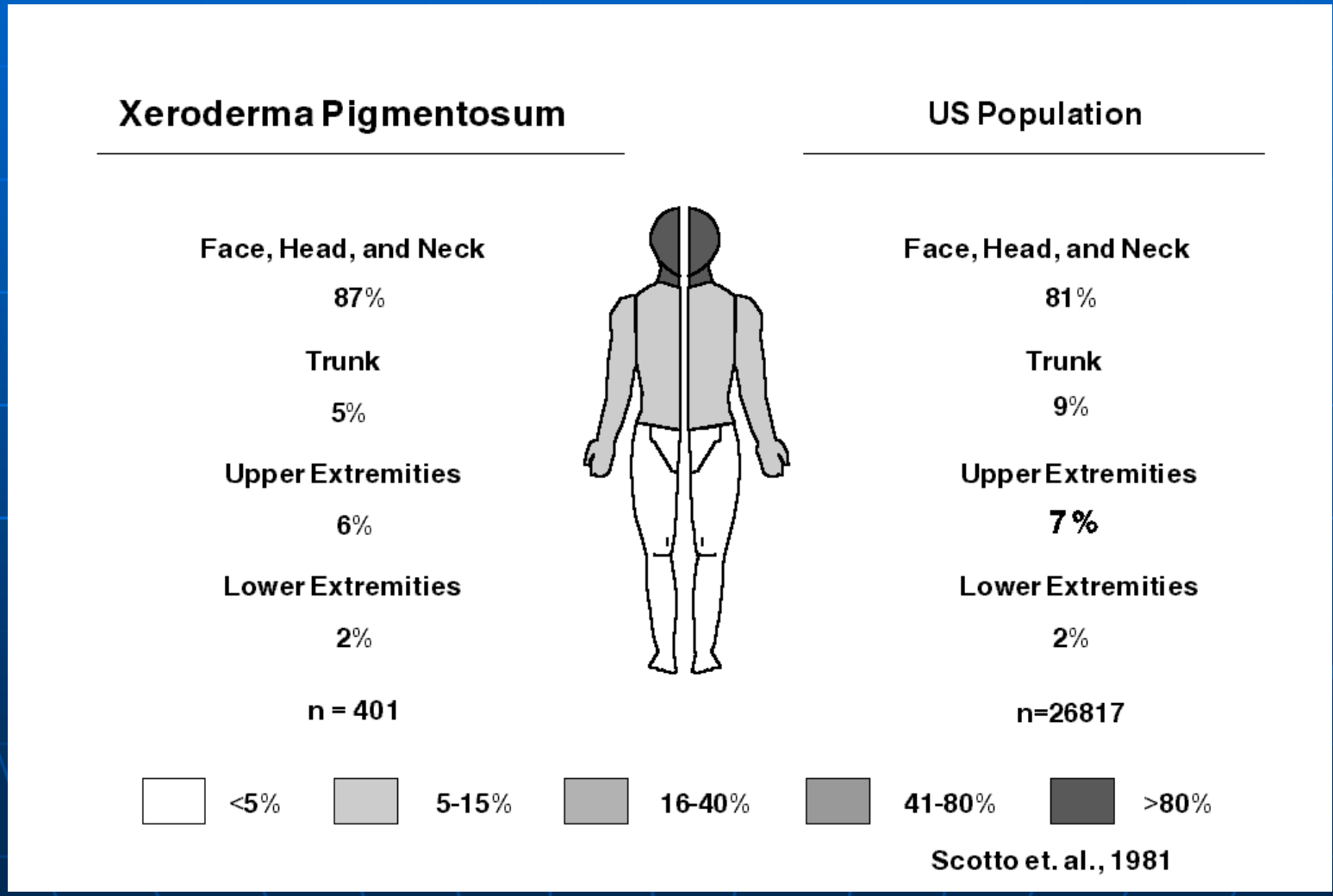
Main question

- What is the role of UV radiation in induction of melanomas in XP patients?

Puzzling relationship between UV exposure and melanoma

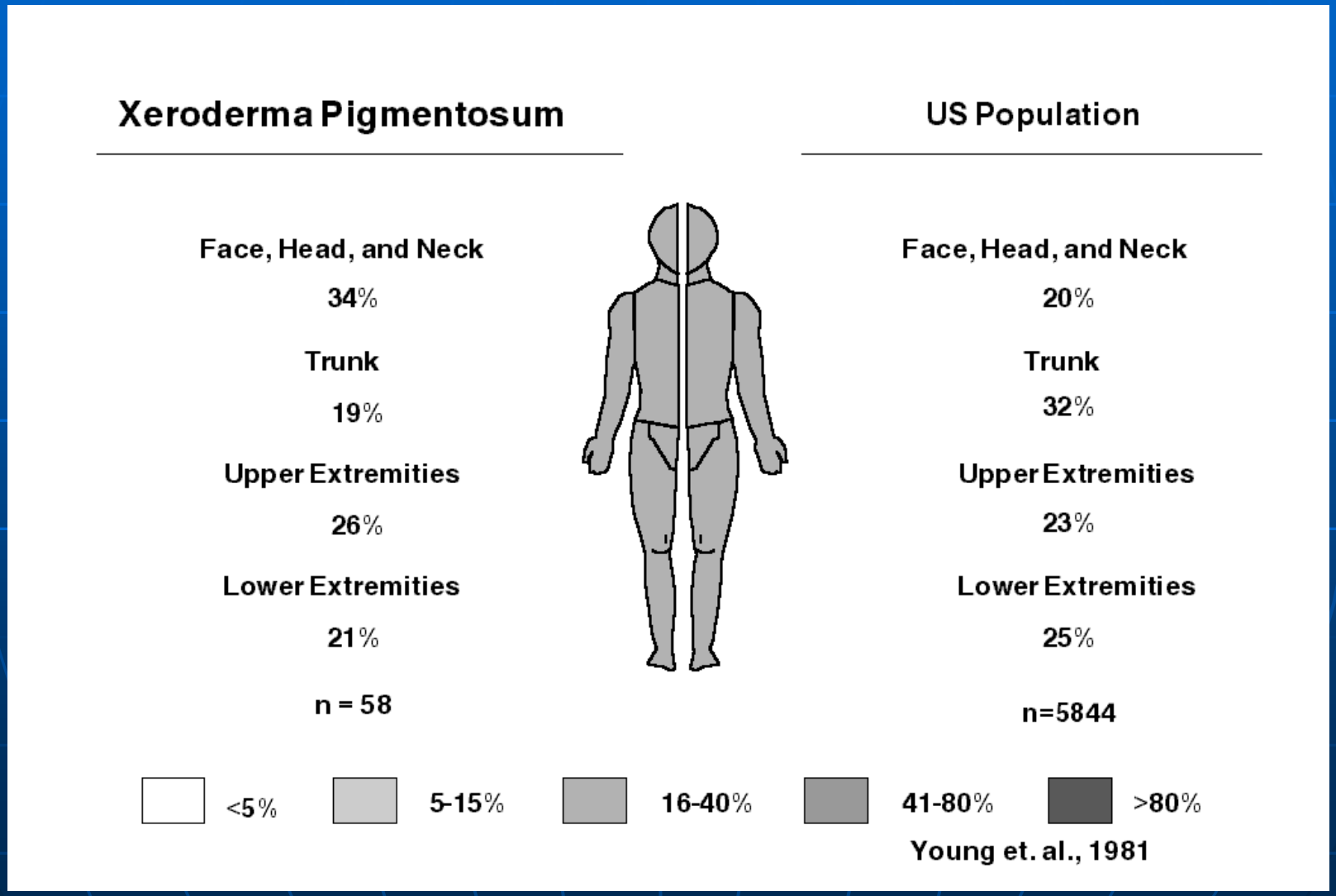
- The site distribution of melanomas is different from that of BCC and SCC and does not correspond to the most sun exposed areas of the body (face, head and neck)

Similar sites of BCC and SCC in XP and normals



Kraemer et al, Arch Dermatol 130: 1018 (1994)

Similar sites of melanoma in XP and normals



Kraemer et al, Arch Dermatol 130:1018 (1994)

Puzzling relationship between UV exposure and melanoma

- The site distribution of melanomas is different from that of BCC and SCC and does not correspond to the most sun exposed areas of the body (face, head and neck)
- People with intermittent intense sun exposure have greater melanoma risk than people with constant exposure such as farmers and sailors

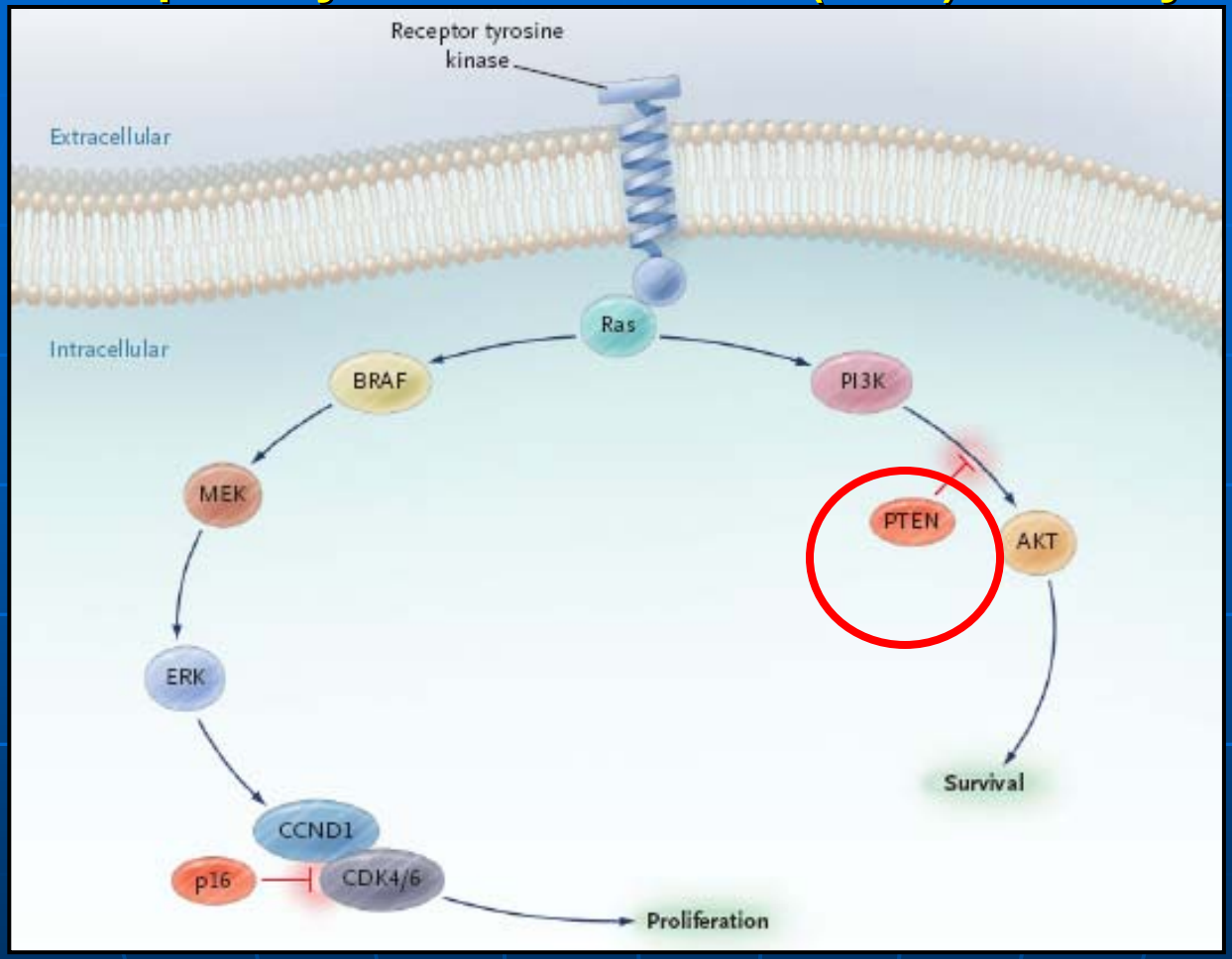
Approach I

- UV radiation results in DNA damage at sites of adjacent pyrimidines (for example TT, CC, TC) forming photoproducts.
- XP patients have defective DNA repair and a 1000-fold increase in melanomas.
- Unrepaired UV photoproducts may result in characteristic mutations (for example C to T mutations).
- Mutations in tumor suppressor genes may result in inactivation leading to cancer.

Approach II

- PTEN tumor suppressor gene has been found to be mutated in many cancers.
- Analysis of base substitution mutations in the PTEN gene in melanomas may provide evidence for UV induction of the mutations and thereby demonstrate a role of UV in causation of melanomas.

The Mitogen-Activated Protein (MAP) Kinase and Phosphatidylinositol 3' Kinase (PI3K) Pathways

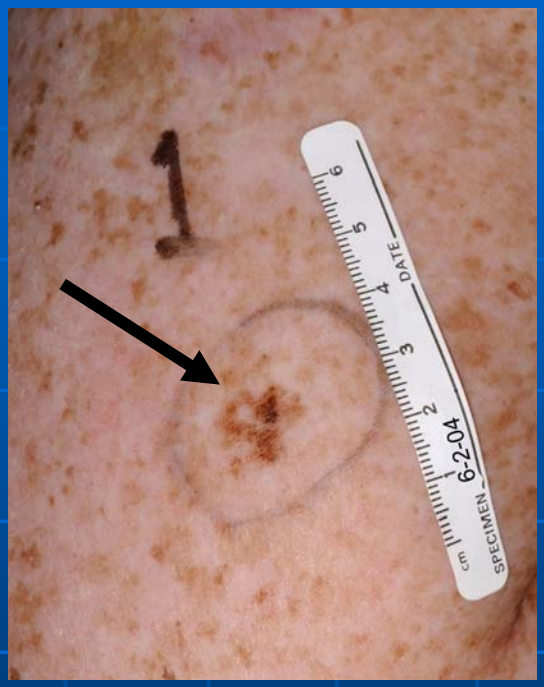


Signals from receptor tyrosine kinases can promote proliferation through the MAP kinase pathway (left branch) and survival through the PI3 kinase pathway (right branch). Phosphatase and tensin homolog (PTEN), a negative regulator of the pathway, may be a somatic target in melanoma.

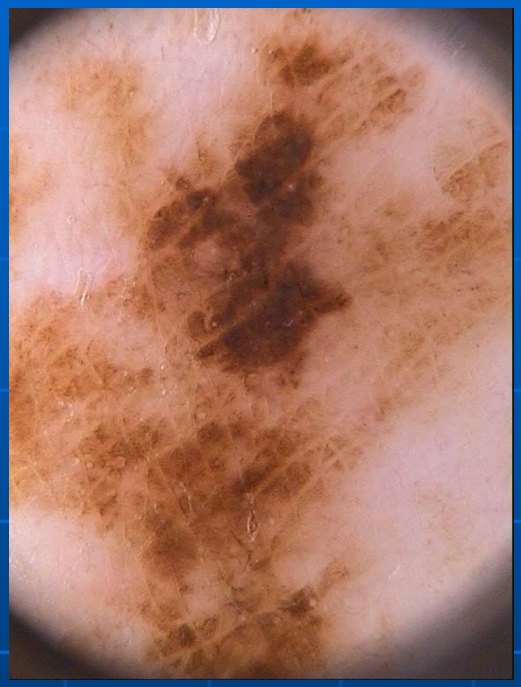
Curtin et al NEJM 353:2135 (2005)

RESULTS

Melanoma *in situ* from 52 y/o patient XP295BE



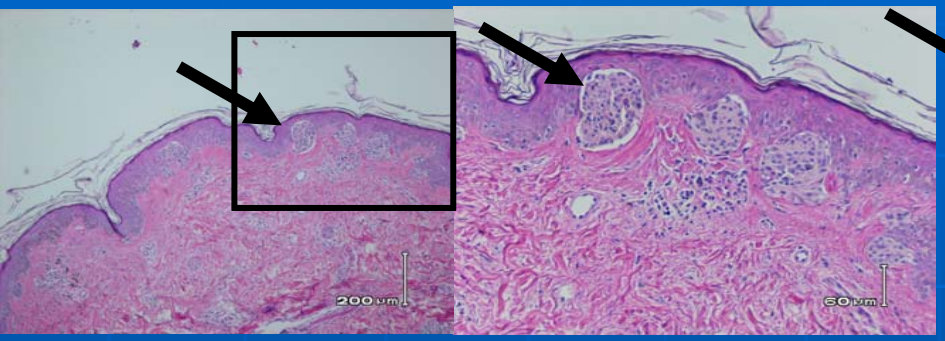
Melanoma on left upper arm.



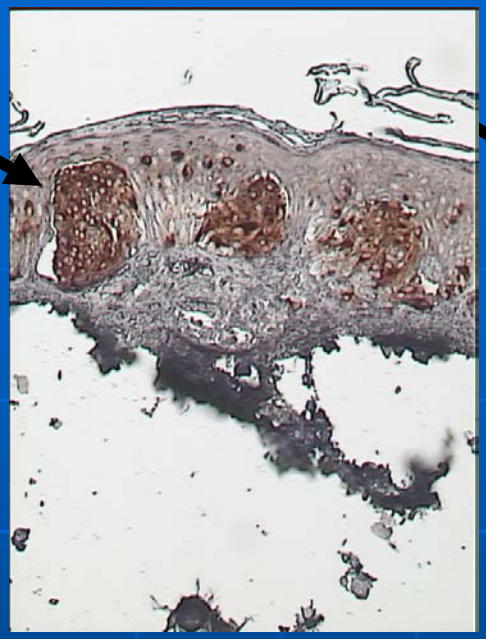
Dermoscopic image showing asymmetrically hyperpigmented lesion (Tumor 1).

PixCell II laser capture microdissector

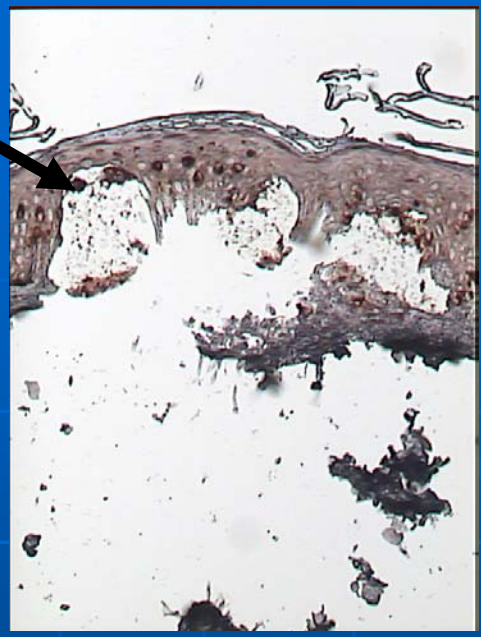
Laser capture microdissection of melanoma tumor 1



Atypical melanocytes are partly arranged in nests.



Melanoma cells expressing Melan-A



After capture of the melanoma cells , the remaining tissue can be inspected and the transfer efficiency of the captured cells can be evaluated.

About 300 melanocytes captured and DNA sequencing performed

High frequency of melanomas with base substitution mutations in the PTEN gene

	Patient	Age/ Sex	CG	Number of Melanomas
1	XP295BE	49/F*	XPC	5 (0)**
2	XP86BE	52/F*	XPC	2 (0)
3	XP376BE	44/F*	XPC	3 (0)
5	XP24BE	29/F	XPC	6 (0)
6	XP29BE	32/M	XPD	18 (5)
7	XP31BE	60/M	VAR	2 (1)
8	XP1BE	45/F	XPC	14 (2)
Tot	8 patients			59 (12)
Freq				100%

8 XP patients had 59 melanomas

*Members of same kindred **Invasive melanoma

High frequency of melanomas with base substitution mutations in the PTEN gene

	Patient	Age/ Sex	CG	Number of Melanomas	Number of melanomas with PTEN mutations
1	XP295BE	49/F*	XPC	5 (0)**	3 (0)**
2	XP86BE	52/F*	XPC	2 (0)	1 (0)
3	XP376BE	44/F*	XPC	3 (0)	2 (0)
5	XP24BE	29/F	XPC	6 (0)	3 (0)
6	XP29BE	32/M	XPD	18 (5)	10 (3)
7	XP31BE	60/M	VAR	2 (1)	0 (0)
8	XP1BE	45/F	XPC	14 (2)	10 (2)
Tot	8 patients			59 (12)	33 (6)
Freq				100%	56%(50%)

56% of the melanomas had PTEN mutations

*Members of same kindred **Invasive melanoma

High frequency of XP melanomas with multiple base substitution mutations in the PTEN gene

Number of mutations per melanoma	1	2	3	4	All
Number of melanomas	19 (58%)	8 (24%)	5 (15%)	1 (3%)	33 (100%)
MM	3 (50%)	1 (17%)	2 (33%)	0	6

42% of the melanomas with mutations had multiple mutations, a feature of UV mutagenesis

Significant frequency of UV type base substitution mutations in the PTEN gene in

Significant increase in UV type mutations compared to expected frequency

	UV type	Not UV type	Total
PTEN sequence (expected frequency*)	746 bp (54%)	641 bp (46%)	1387 bp (100%)

*frequency of dipyrimidines

**P=0.0001

Significant frequency of UV type mutations in PTEN gene in XP melanomas compared to

Significant increase in UV type mutations compared to internal cancers

Number of mutations (obs freq)	UV type	Not UV type	Total
Melanoma of XP	48 (89%)	6 (11%)	54 (100%)
PTEN sequence (expected frequency)	746 bp (54%)	641 bp (46%)	1387 bp (100%)

*from Sanger database

**P=0.0001 vs Melanoma

PTEN mutations frequently change amino acids in XP melanomas

		UV type	Not UV type	Total
AA c	Nonsense (stop)	2 (4%)	0	2 (4%)
				(4%)
				(61%)
	Total	35 (65%)	2 (4%)	37 (69%)
No AA change		13 (24%)	4 (7%)	17 (31%)
Total		48 (89%)	6 (11%)	54 (100%)

37 (69%) of the 54 mutations change the amino acid

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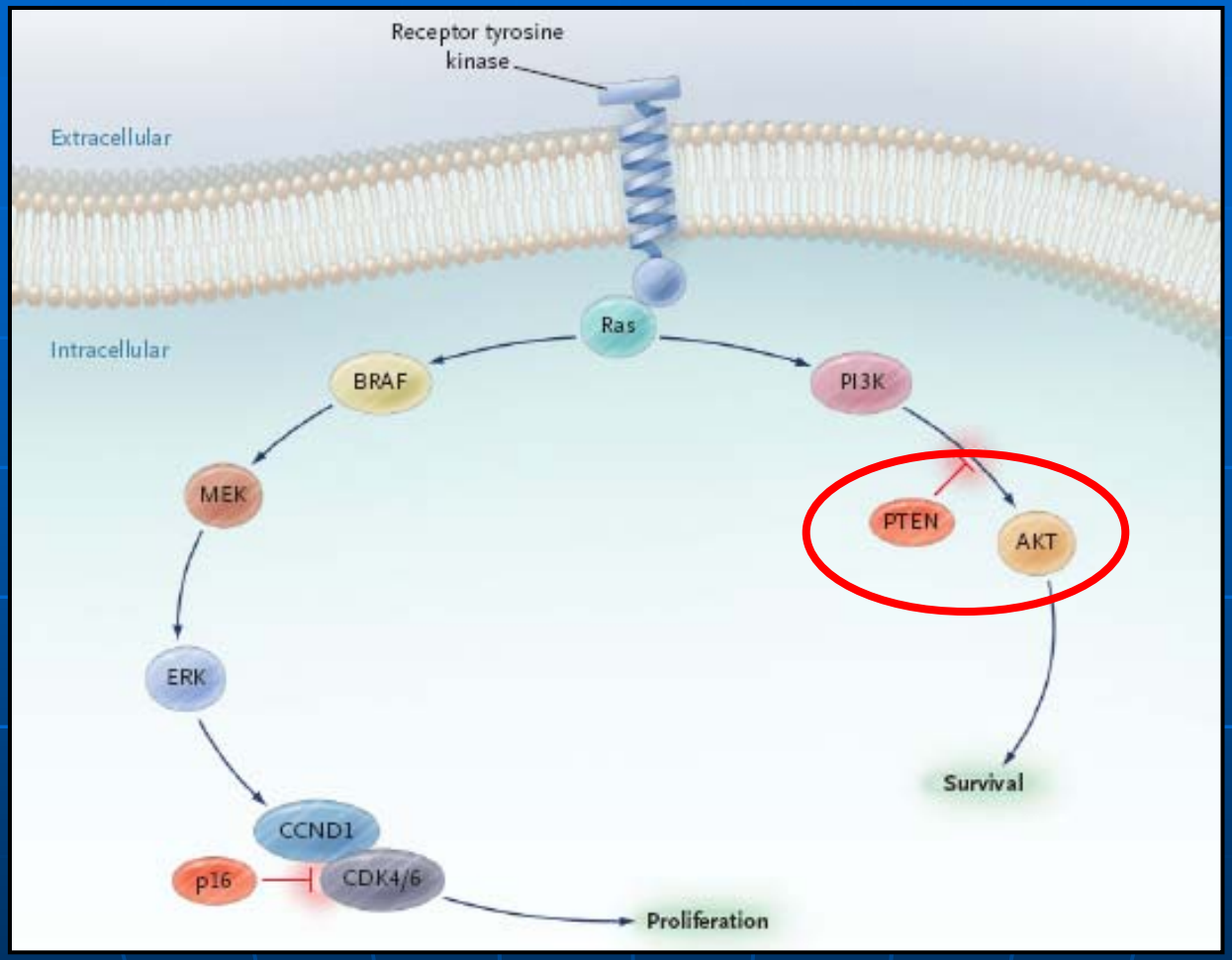

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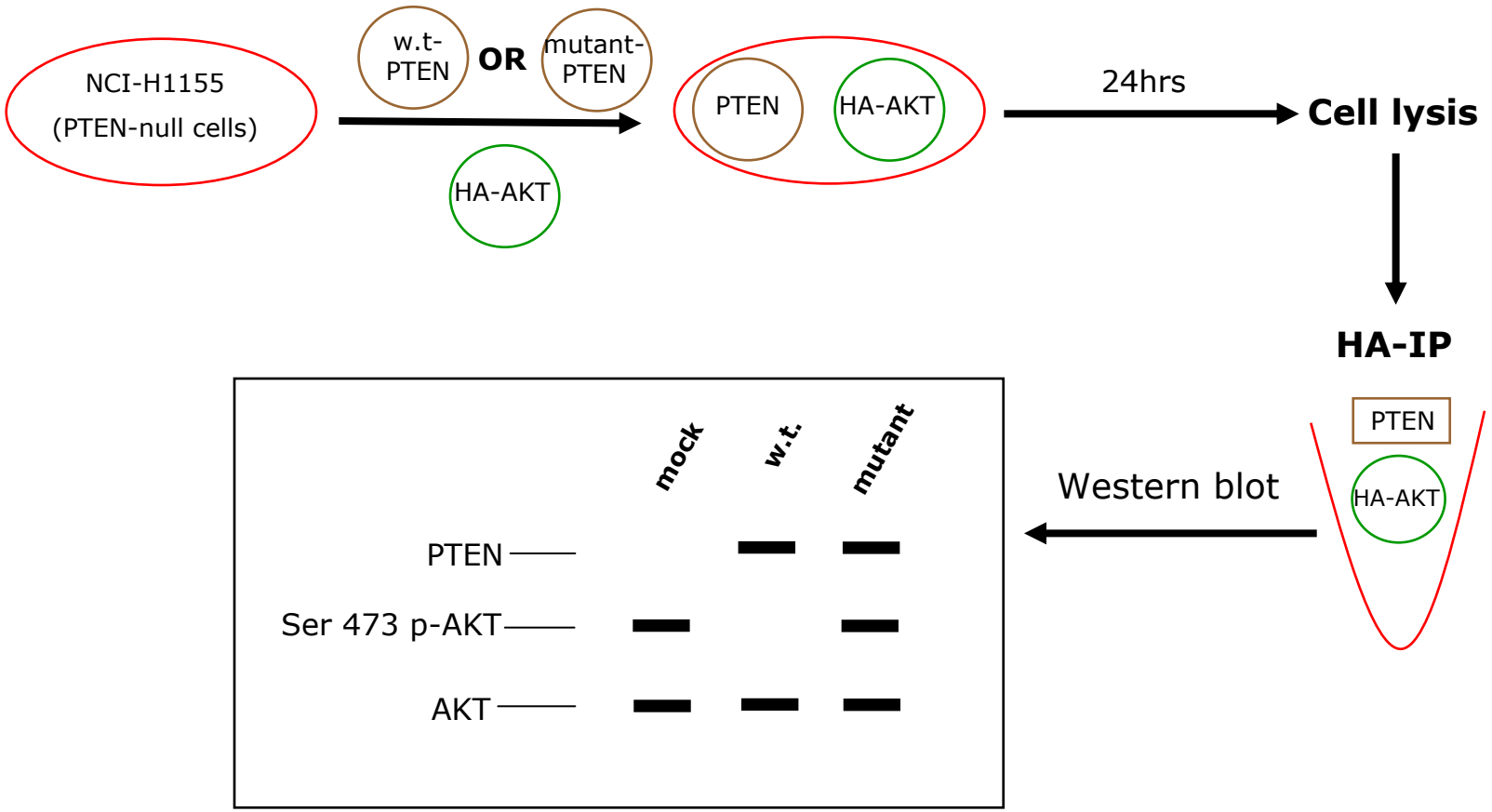
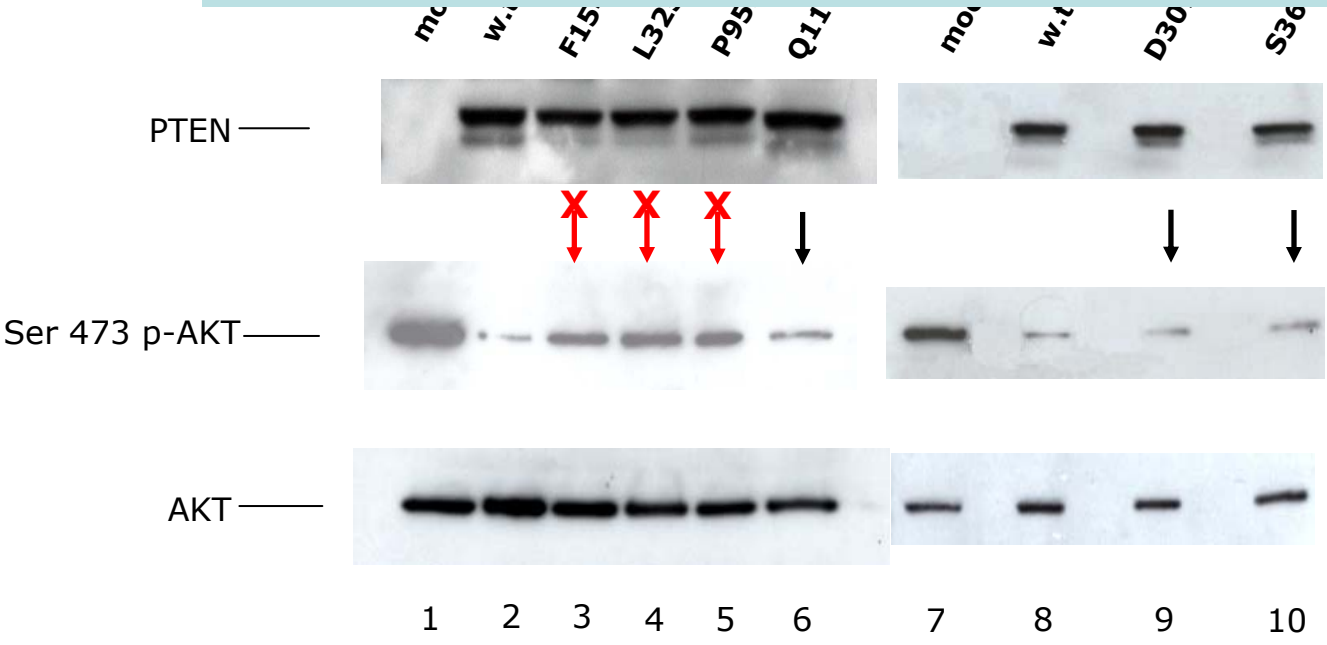
Functional Assay of Phosphorylation of AKT by PTEN Mutants



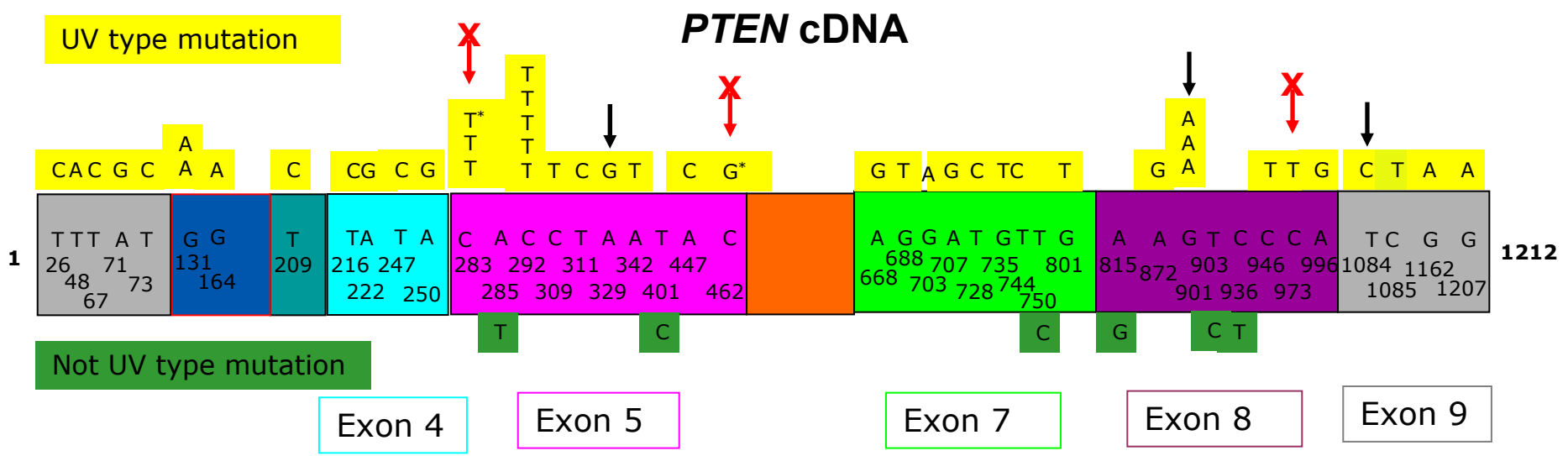
Functional assay of phosphorylation of AKT by selected PTEN mutants

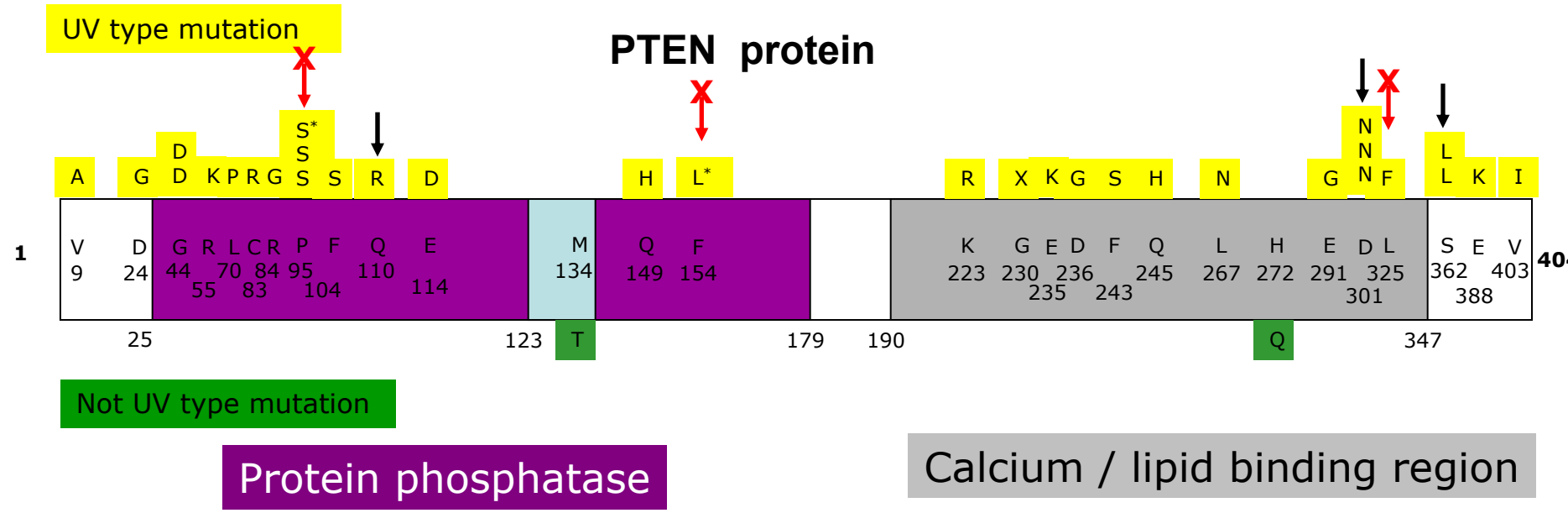
3/6 (50%) of the mutations reduce PTEN function



Western blot of PTEN, P-AKT and AKT

UV-TYPE MUTATIONS IMPAIR PTEN FUNCTION

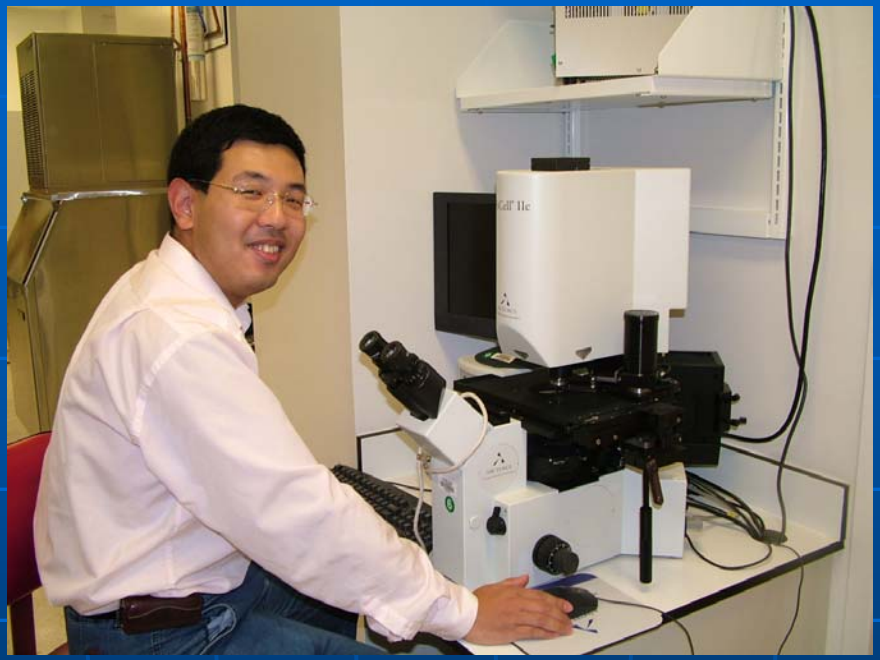




Conclusions

- Ultraviolet radiation damage plays a direct role in induction of PTEN mutations in melanomas in XP patients
- Frequent PTEN mutations in *in situ* melanomas indicate that it is an early event in melanoma induction
- The variety of mutations indicate that each melanoma arose independently
- These mutations frequently altered the PTEN amino acid sequence
- Some UV-type mutations impaired PTEN function

These data provide solid mechanistic support for UV protection for prevention of melanoma.



Thank you!

Wang et al PNAS 106: 6269 (2009)