Role of BARD1 in cell life and death

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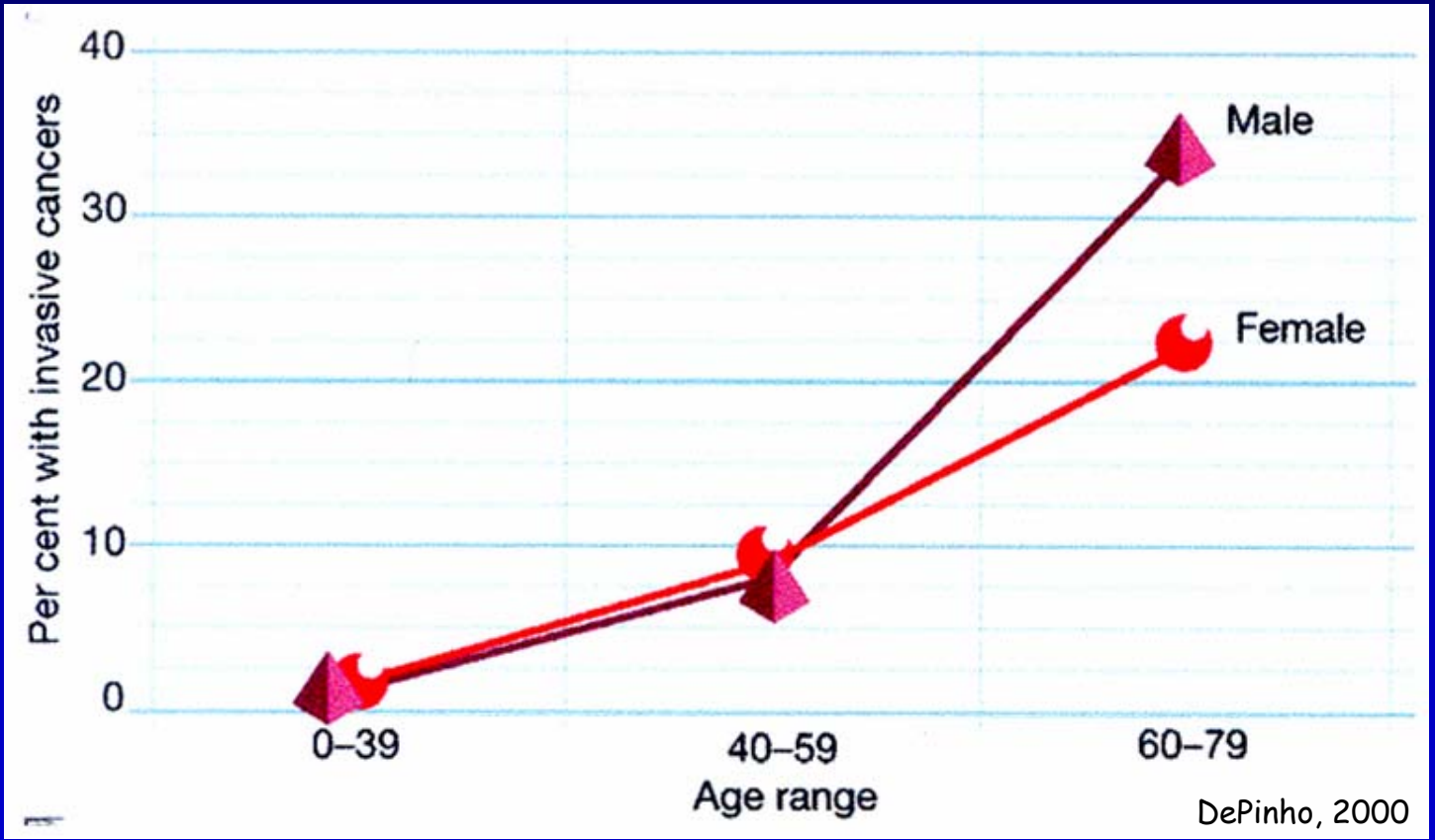
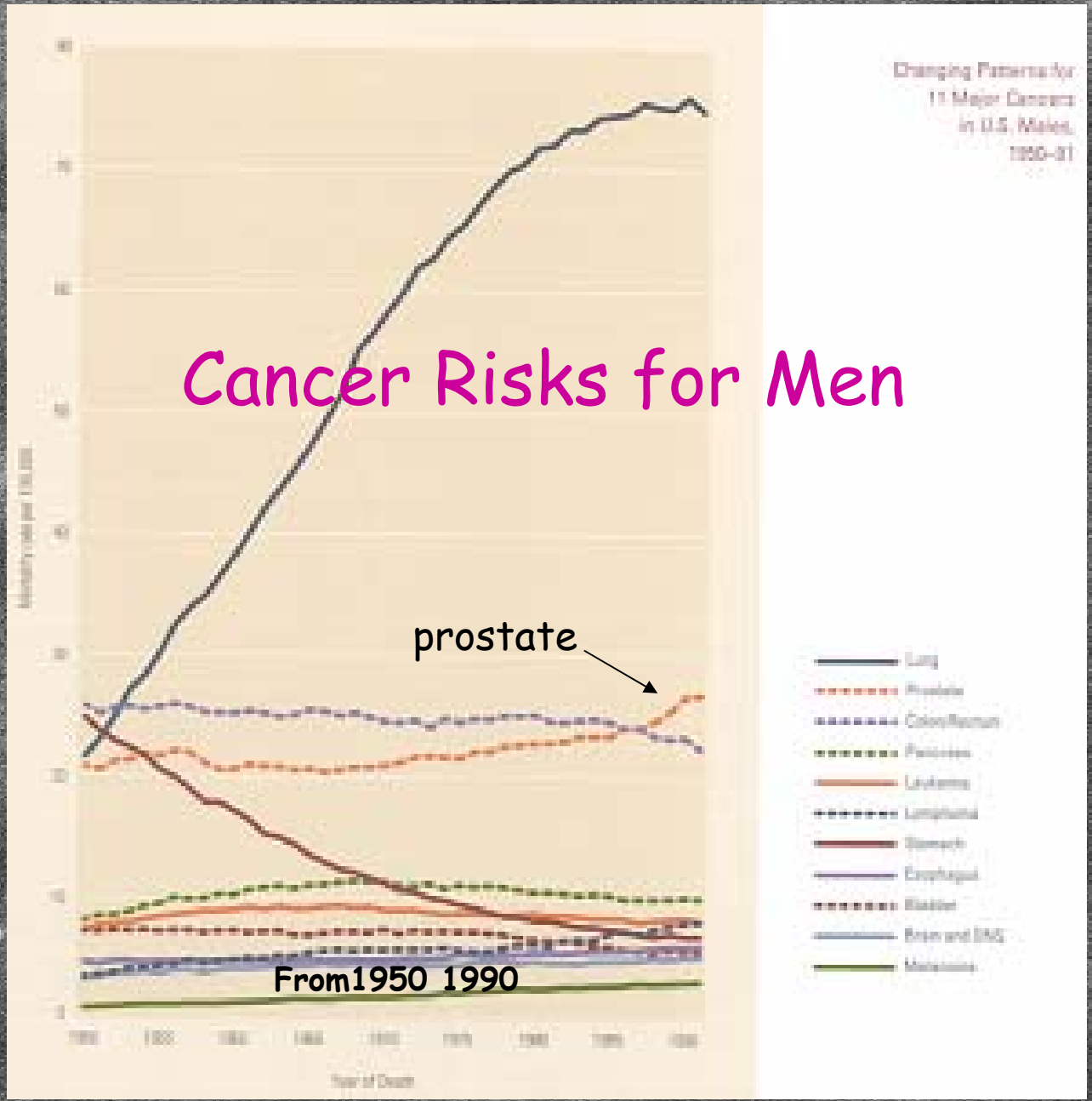
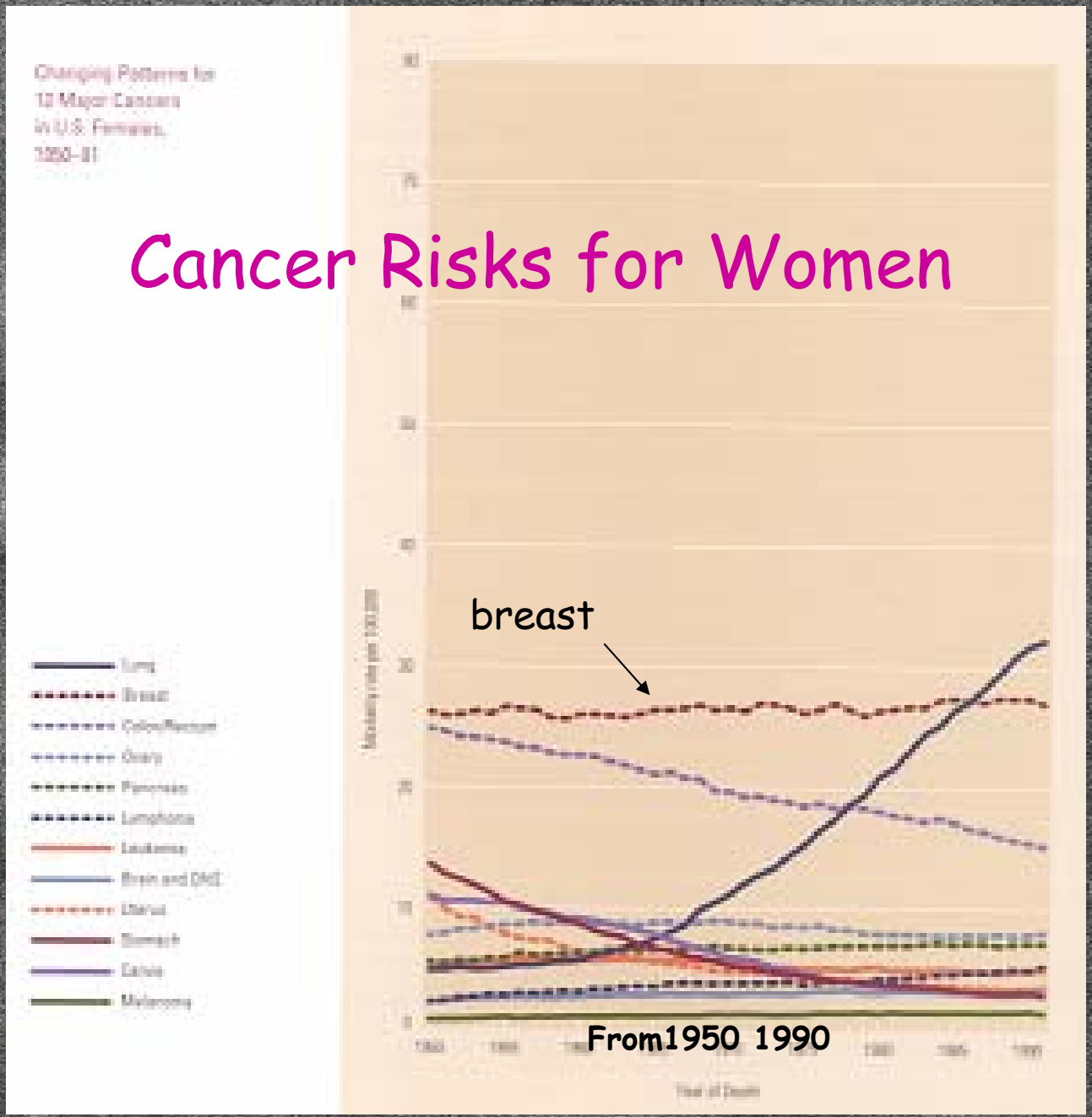
Age is the biggest risk factor for cancer



Cancer Risks for Men

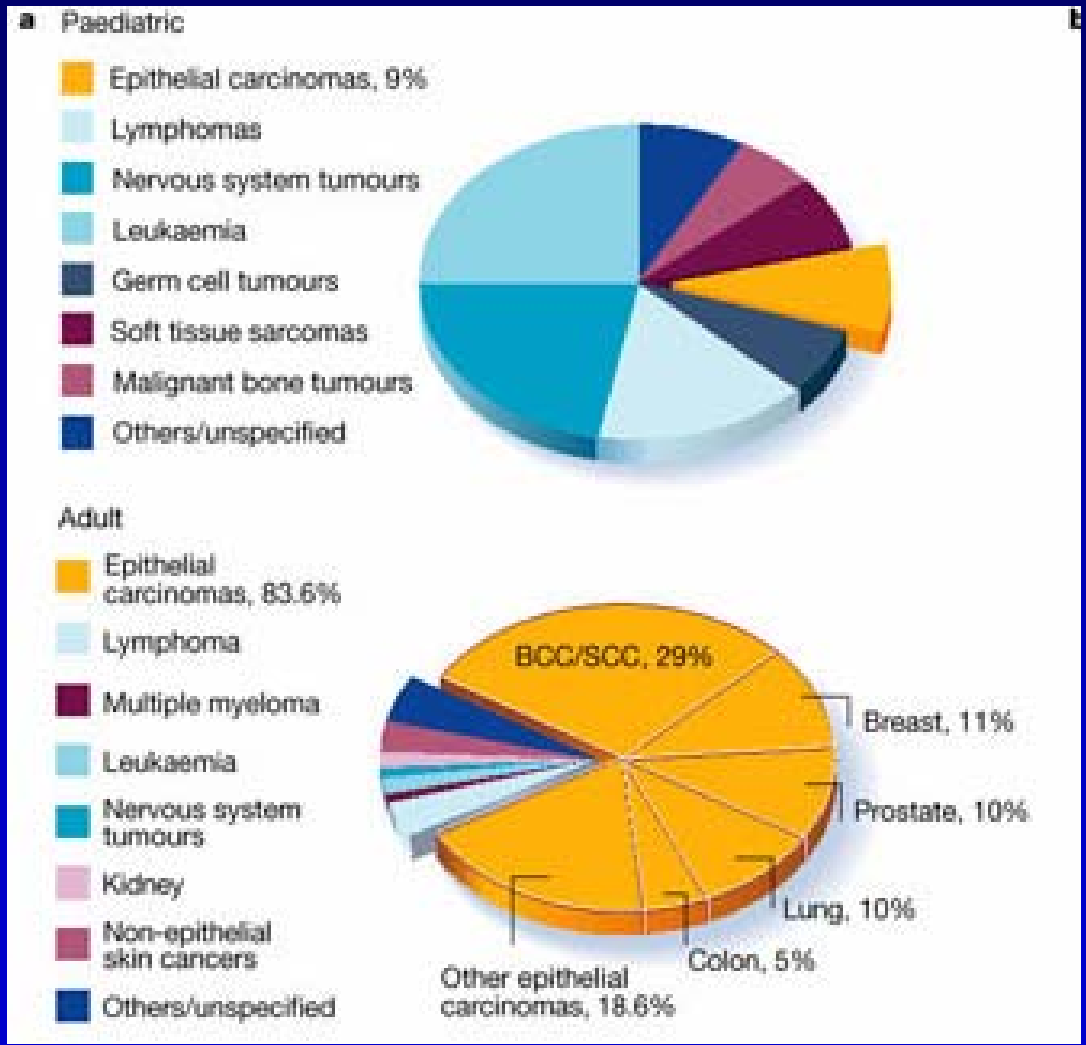


Cancer Risks for Women



Cancers of old age are different from young age cancers

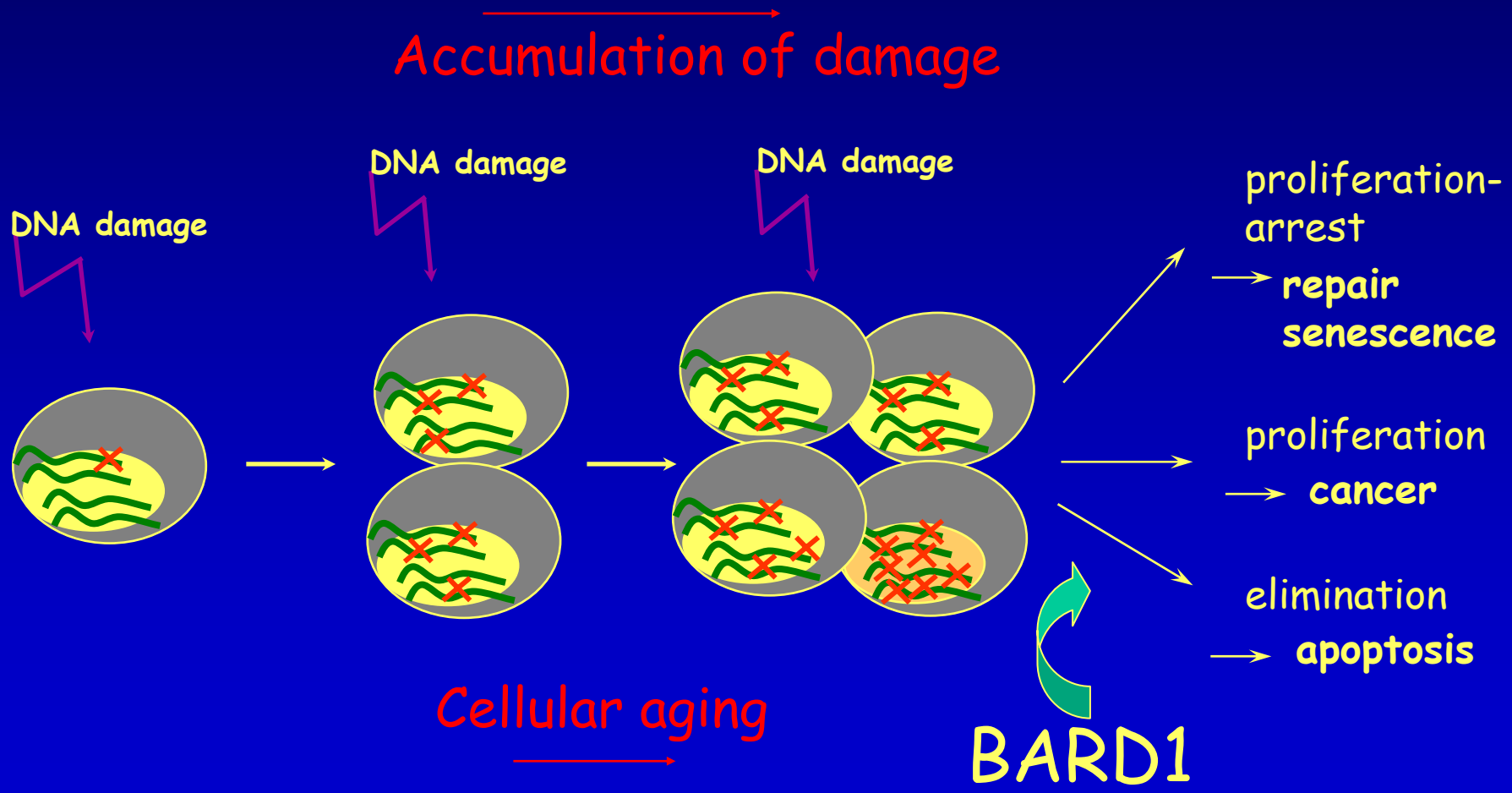
Cancer in old age has a different face than cancer in young age




What could link epithelial cell derived cancers to aging?

BARD1, not just another cancer predisposition gene

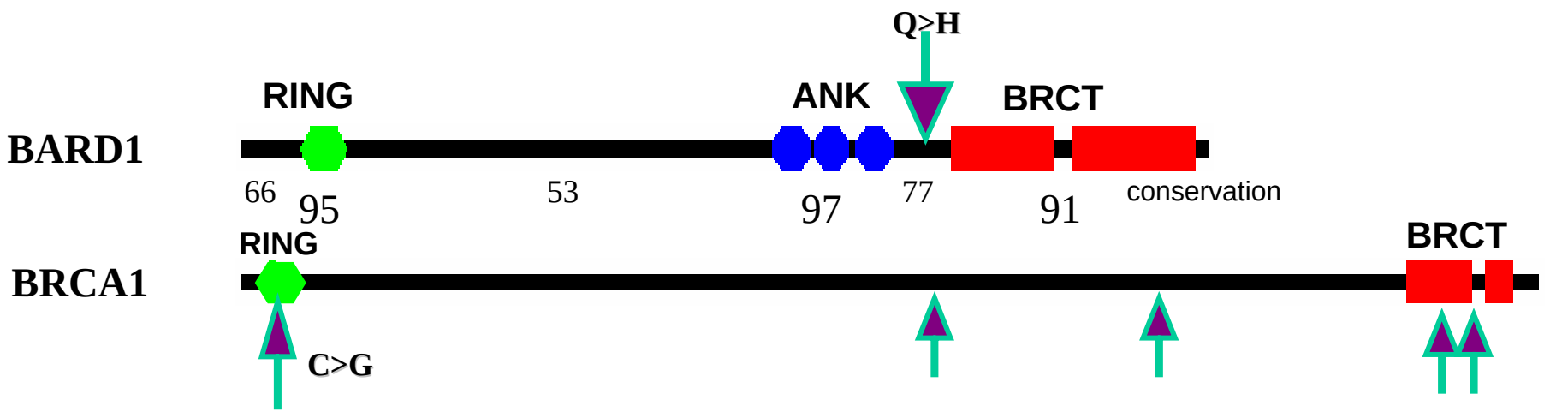
Common pathways for cancer and aging?



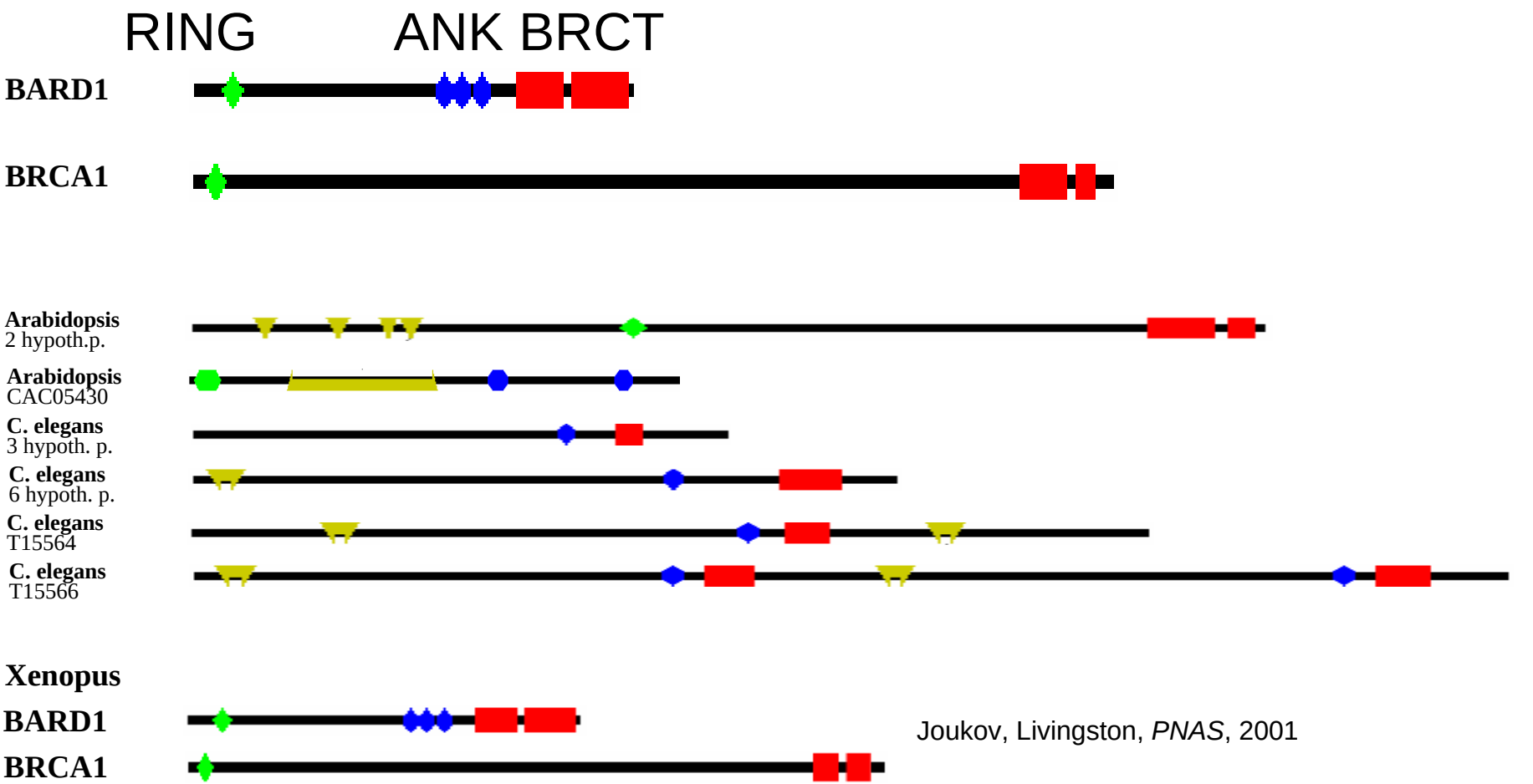
Summary

- BARD1 structure
- BARD1 repression studies
- BARD1 overexpression studies
- BARD1 dynamic localization
- BARD1 induced upon stress
- Non-correlated expression of BARD1 and BRCA1
- Role in spermatogenesis
- BARD1 upregulation upon stress
- Role in tumorigenesis
- BARD1 a tumor antigen
- BARD1 in cancer vaccine and genetherapy

Conserved structures in BARD1 and BRCA1

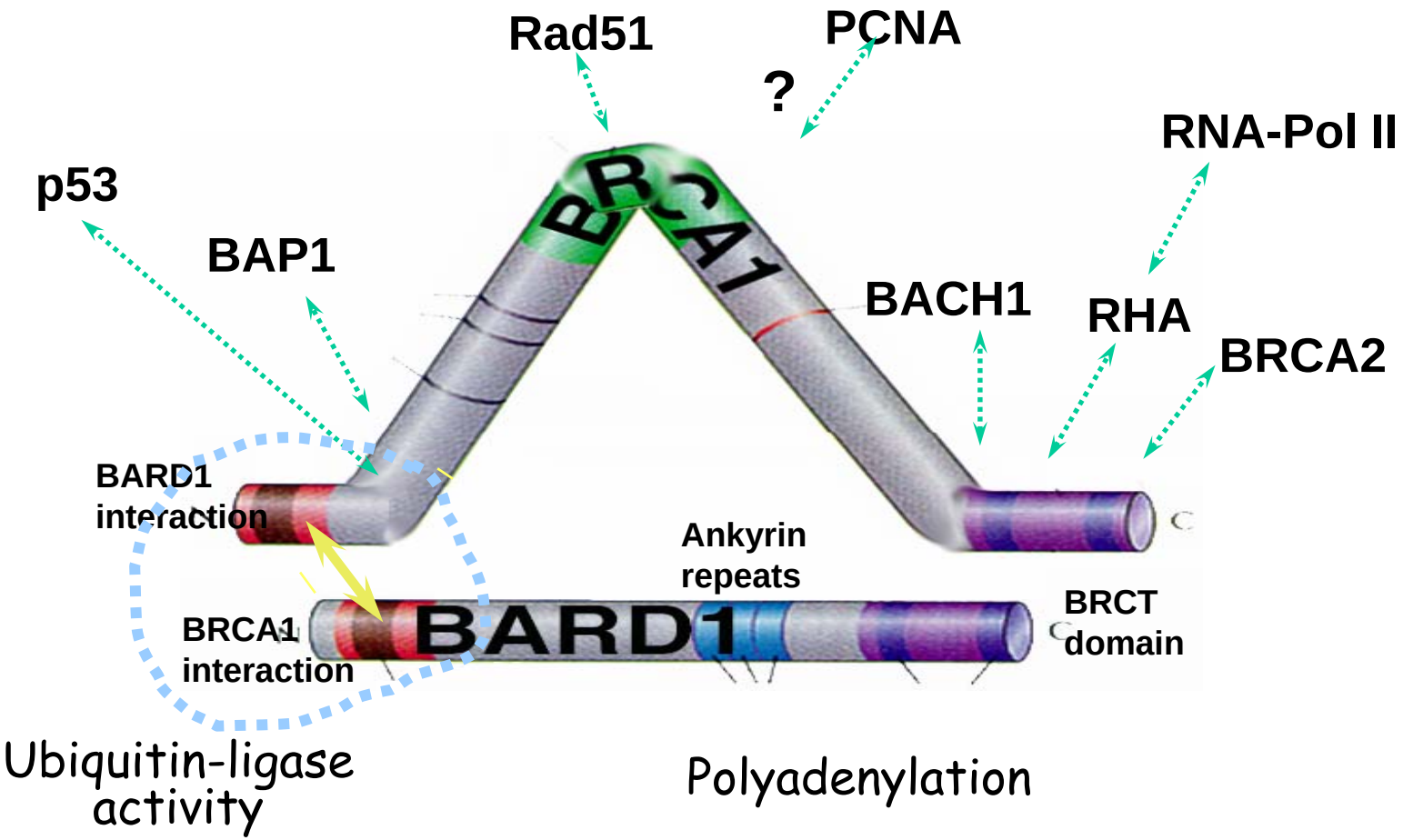


No one like BARD1

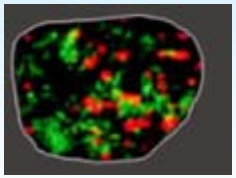


Joukov, Livingston, *PNAS*, 2001

BARD1- BRCA1 heterodimer

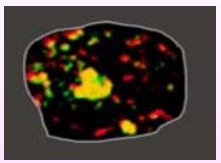


A



Replication?
S-phase dots

HU/UV



DNA REPAIR:
PCNA/Rad51
co-localization
[Jin et al., 1997;
Scully et al., 1997]

B

BARD1

BRCA1

Pol II/Holo

Transcription:
Pol II/Holo Interaction
[Scully et al. 1997}

DNA
damage?

BARD1

BRCA1

TRANSCRIPTION
BLOCK?
Ubiquitin ligase
activity
[Parvin 2001]

C

BRCA1

BARD1

CstF-50

mRNA
Processing?

?

HU/UV

INHIBITION OF
mRNA
processing
[Kleiman &Manley,
2000, 2001]

D

BARD1

Bcl-3

NF-kB

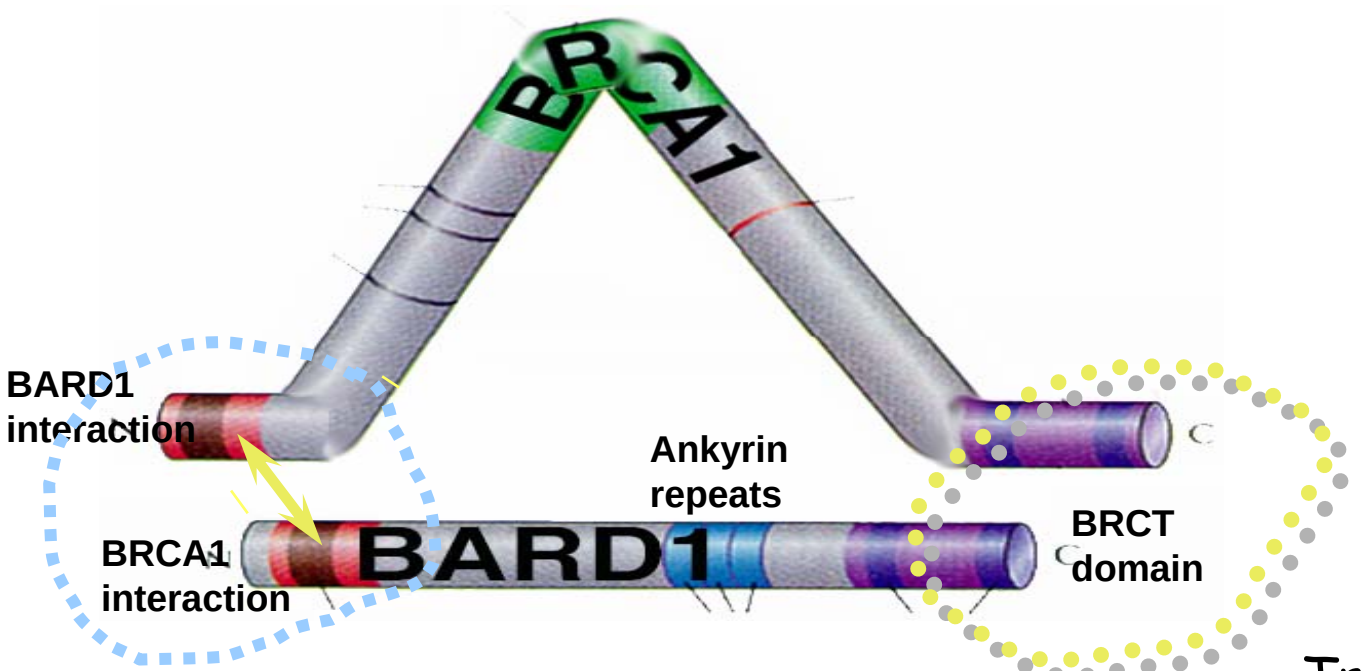
Regulation of
transcription?

?

MODULATION
of NF-κB
activity?
[Dechend et al. 1999]

Does tumor suppressor function of BARD1 depend on BRCA1-BARD1 heterodimer?

BARD1- BRCA1 Heterodimer



Ubiquitin-ligase activity

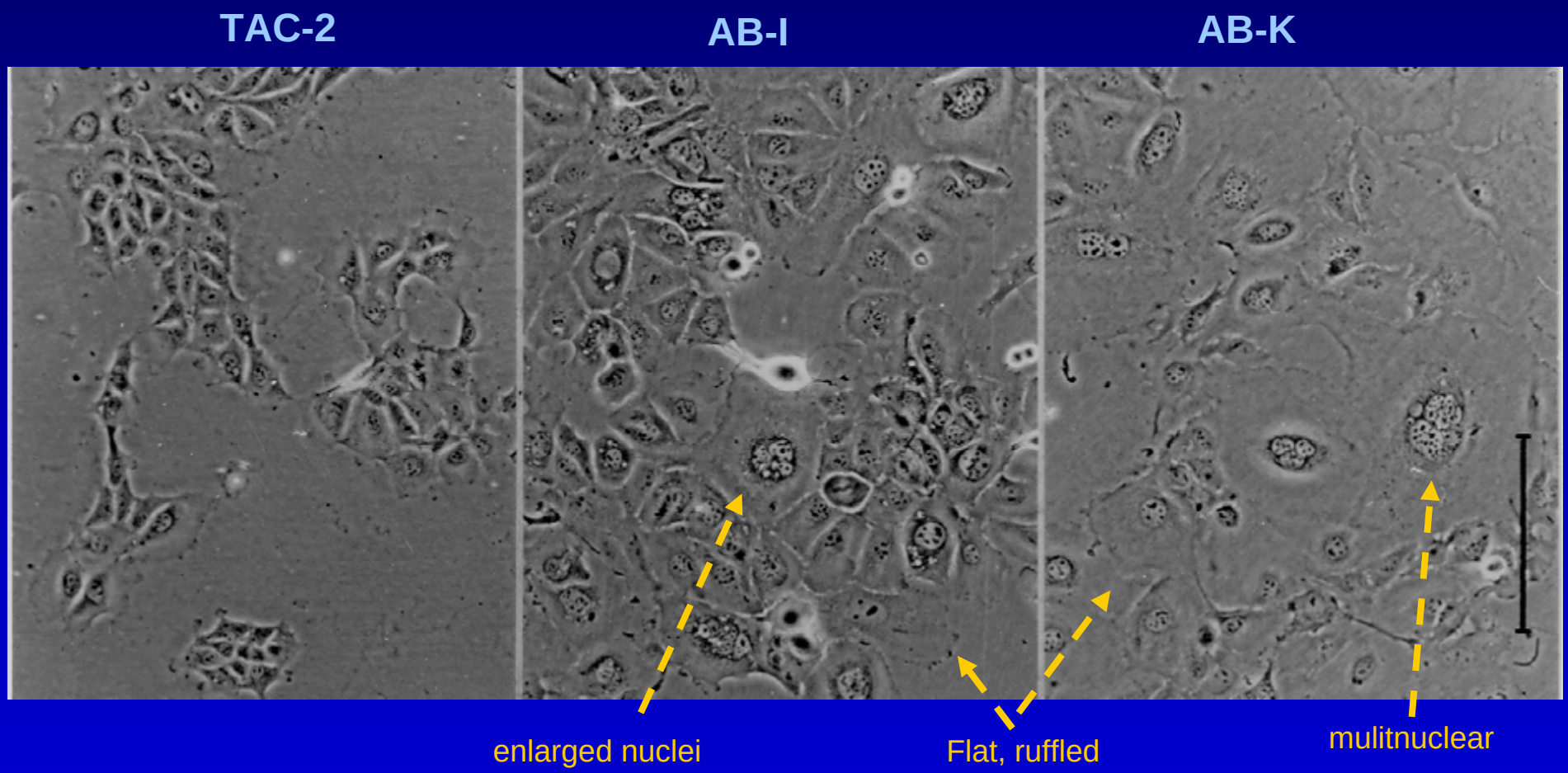
Polyadenylation

Transcription Activation?

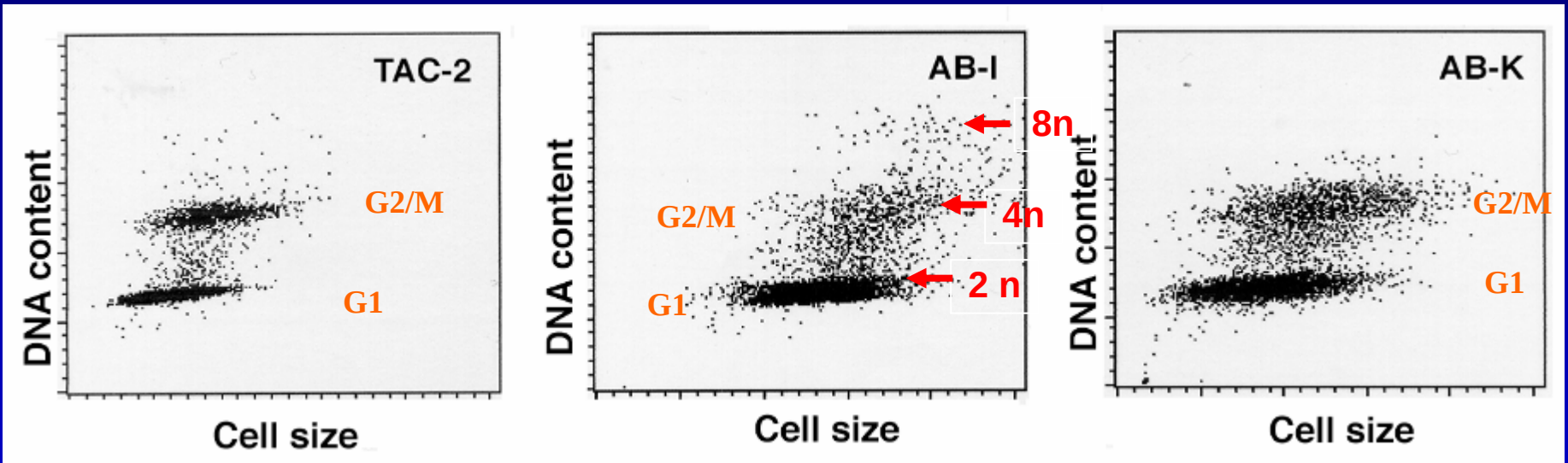
BARD1 repression in vitro

- mouse mammary epithelial cells
- repression of BARD1 with antisense and ribozyme constructs
- analysis of phenotype: MORPHOLOGY, CELL CYCLE, MORPHOGENETIC PROPERTIES

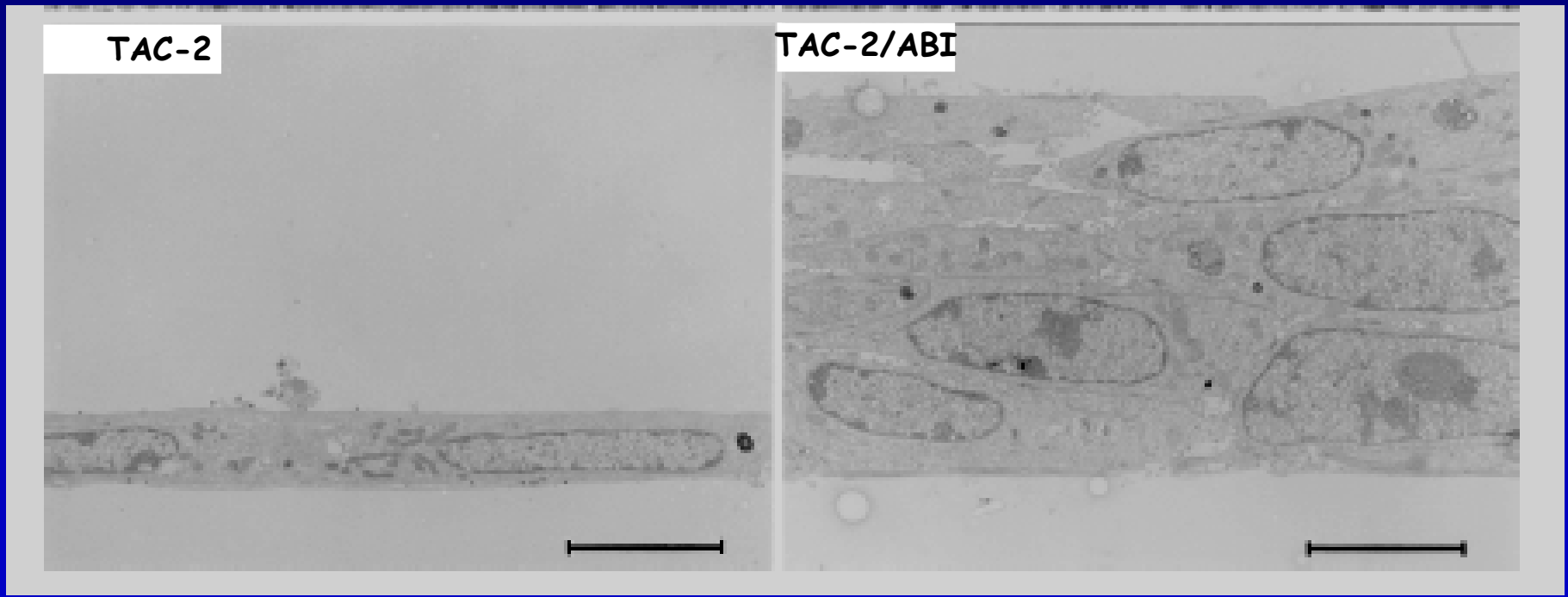
Altered morphology of *BARD1* antisense and ribozyme expressing cells



Genomic instability in *BARD1*-repressed cells



BARD1 repression results in loss of contact inhibition of growth

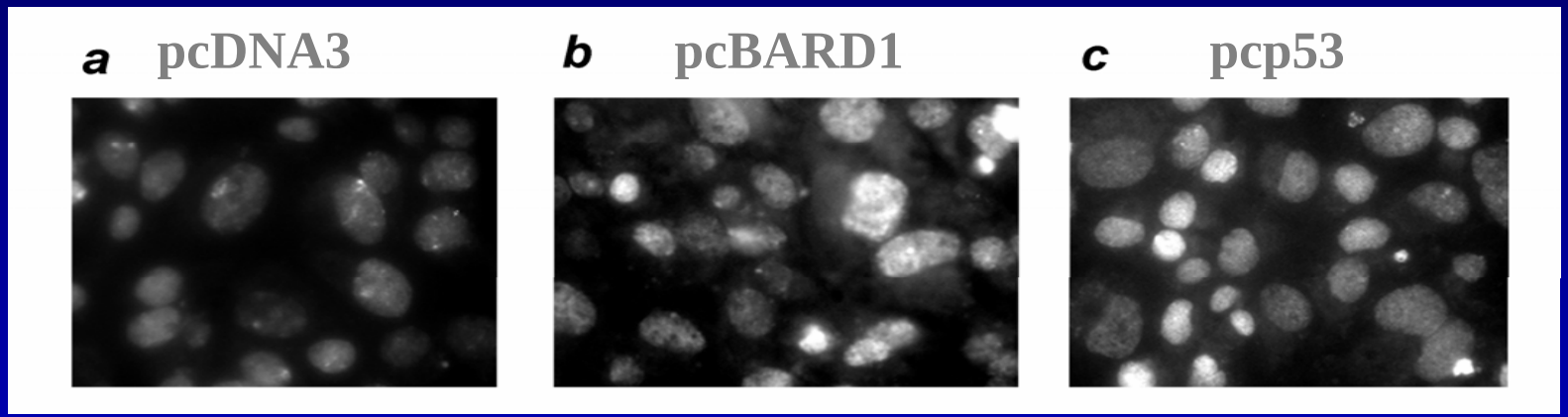


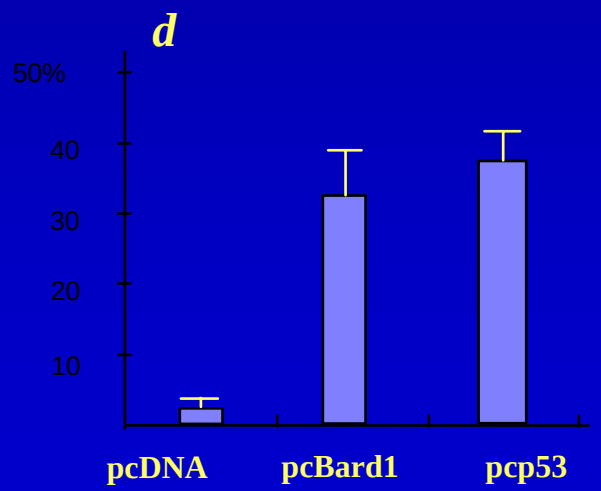
BARD1 overexpression studies

- Constitutive expression of mouse BARD1 cDNA
- Based on the model of functional BARD1-BRCA1 heterodimer, overexpression should have no phenotype unless BARD1 has a BRCA1 independent function
- Result:
  - cells over-expressing exogenous BARD1 are not viable

BARD1 overexpression leads to apoptosis

TUNEL staining

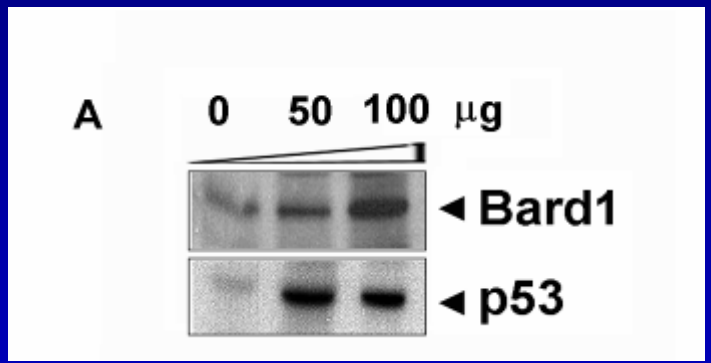




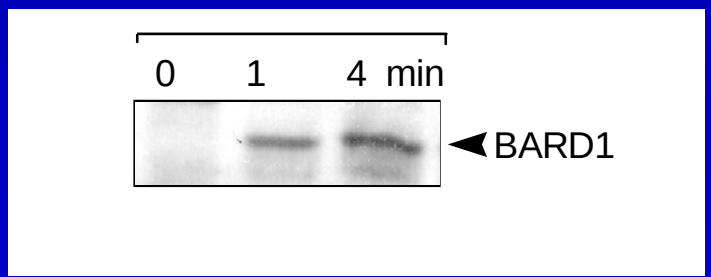
BARD1 is upregulated in response to cellular stress

On the protein level

Doxorubicin
TAC-2 cells

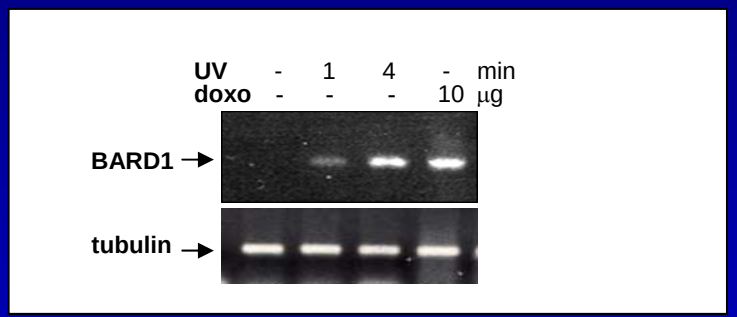


UV
ES cells

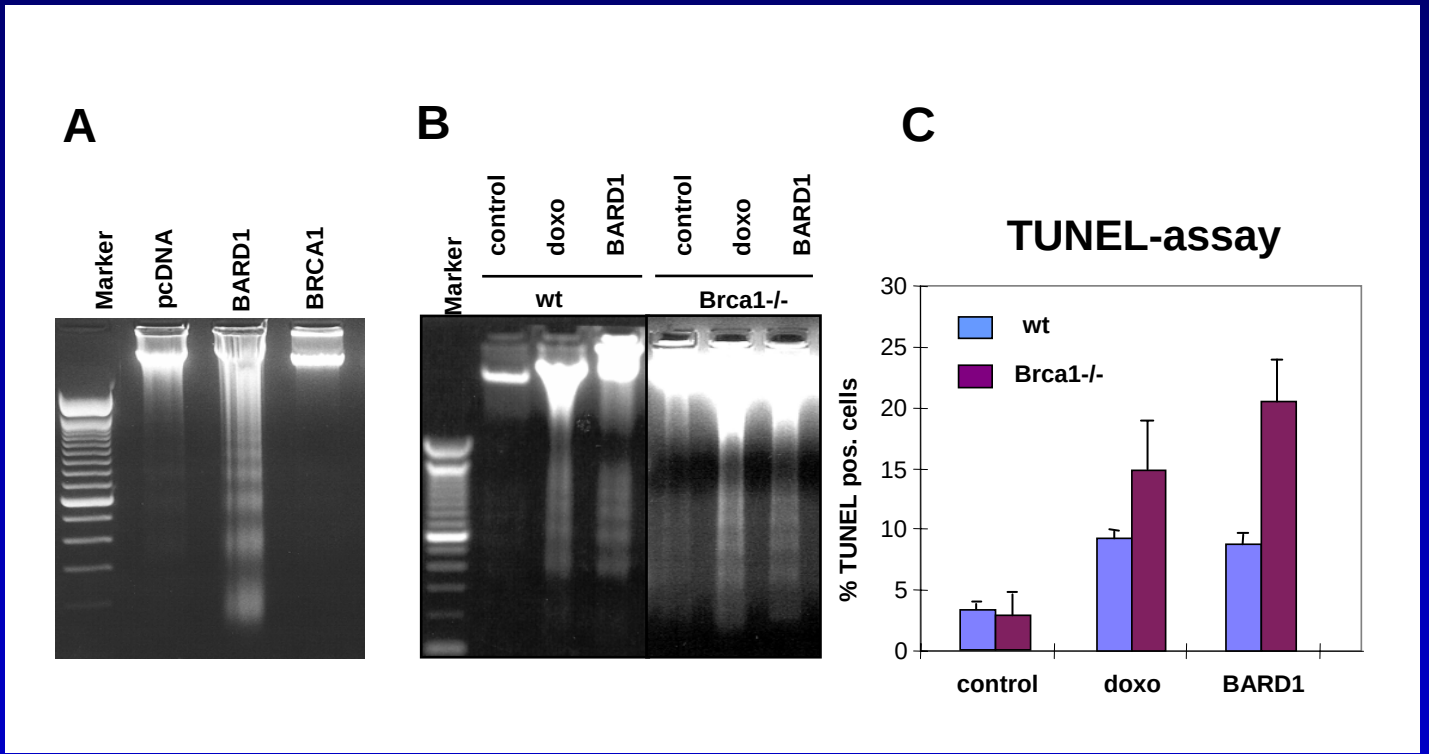


On the mRNA level

RT-PCR



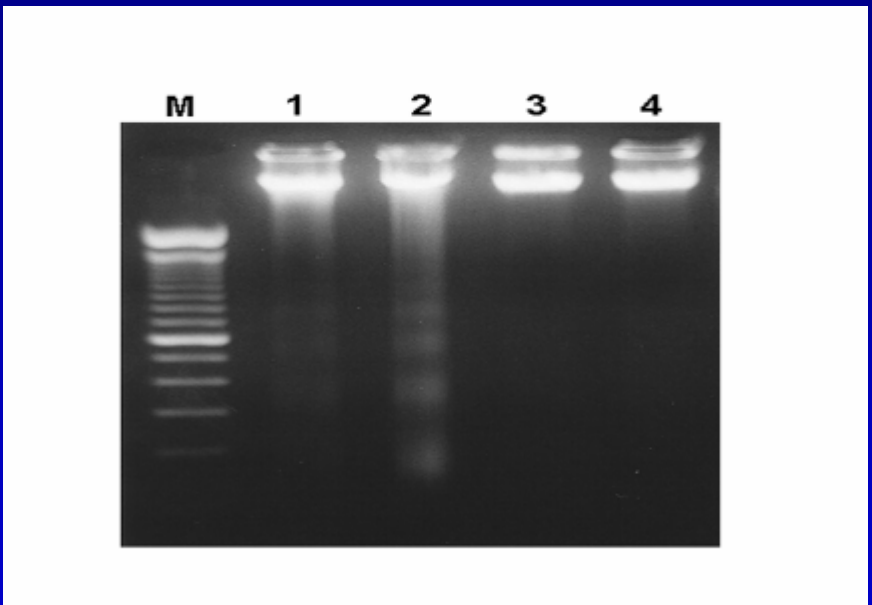
BARD1-induced apoptosis is independent of BRCA1



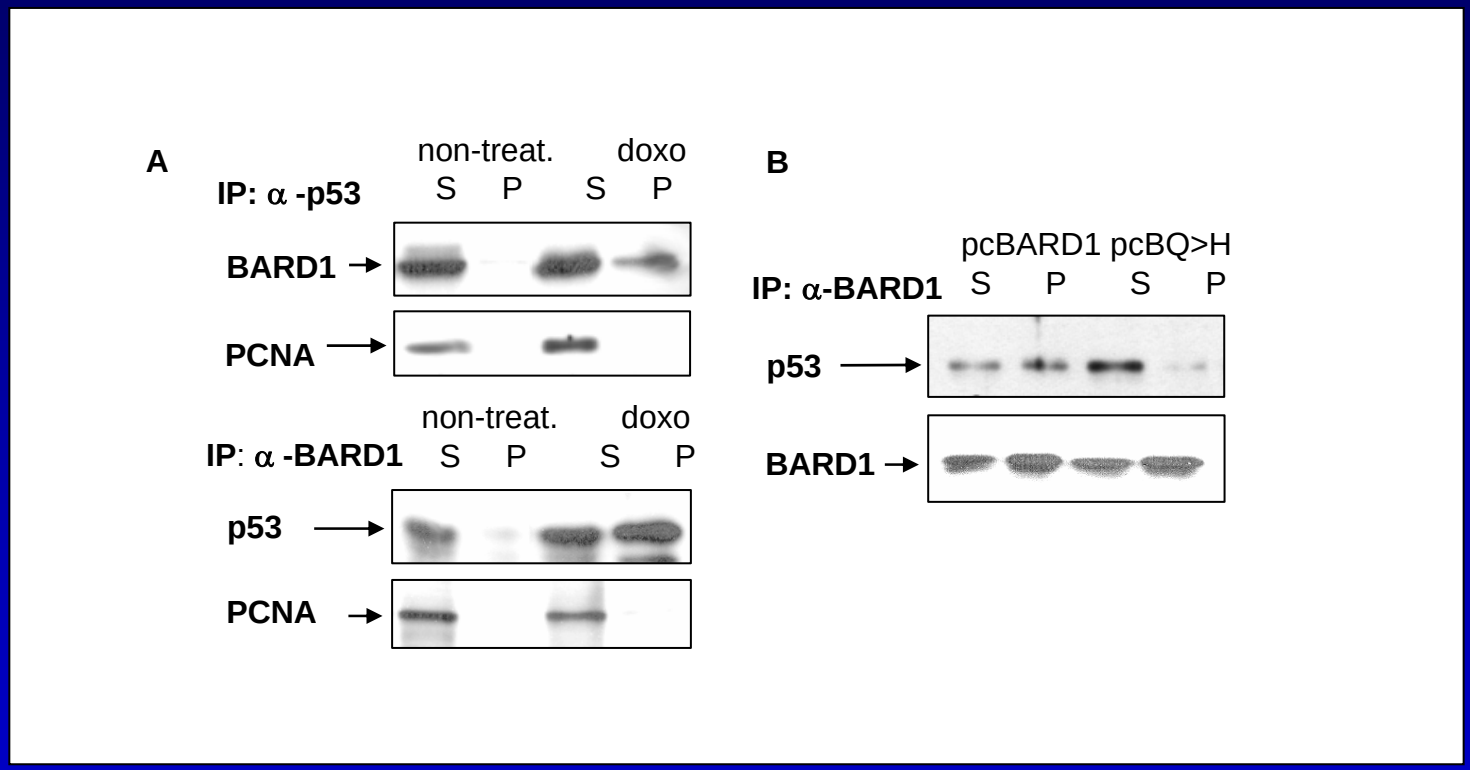
What is the mechanism of BARD1-induced apoptosis ?

BARD1-induced apoptosis mediated by p53

Induction of apoptosis after transfection of BARD1

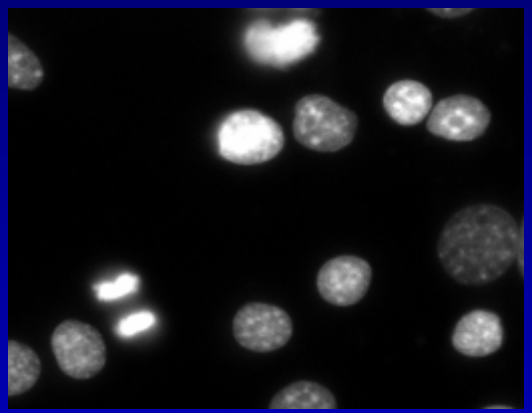


BARD1 stabilizes p53 by direct interaction

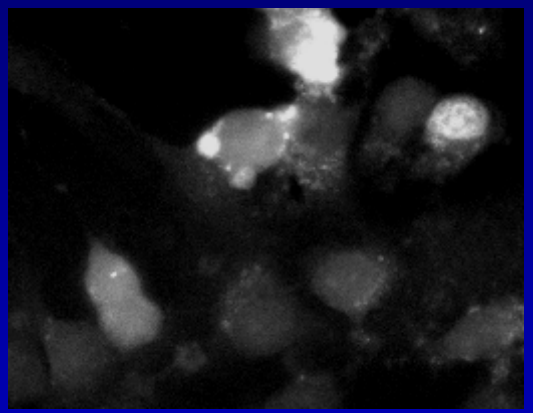


Localization of BARD1 during apotosis

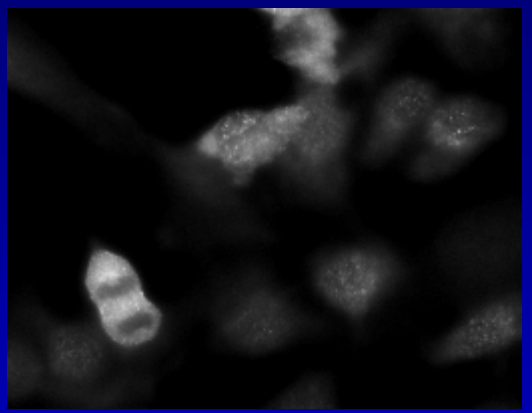
A. nuclei (DAPI)



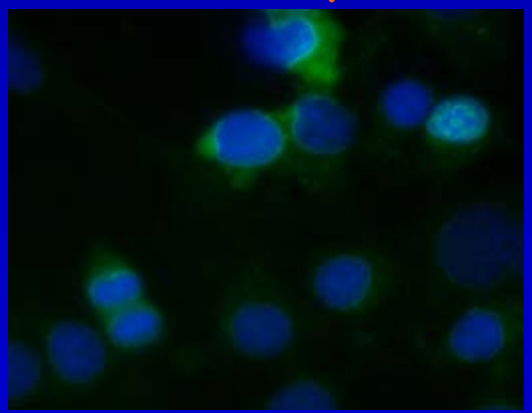
B. BARD-GFP



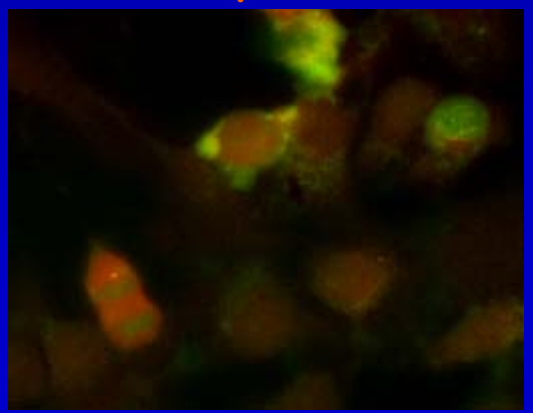
C. Anti-BARD1



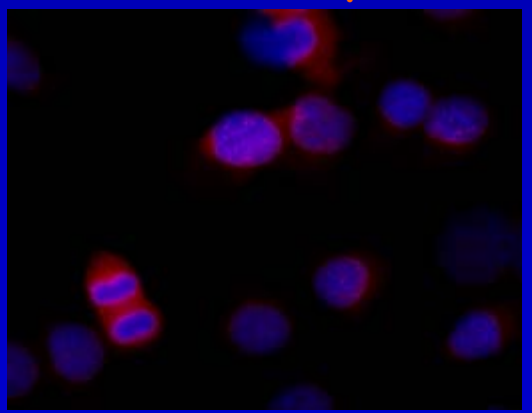
A+B overlay



B+C overlay

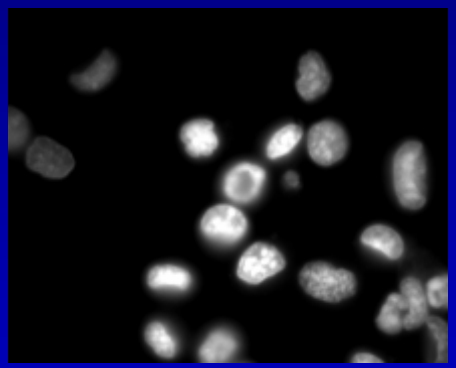


A+C overlay

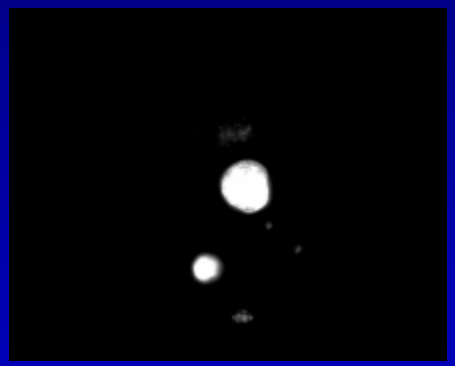


Localization of BARD1-GFP deletion mutant during apoptosis

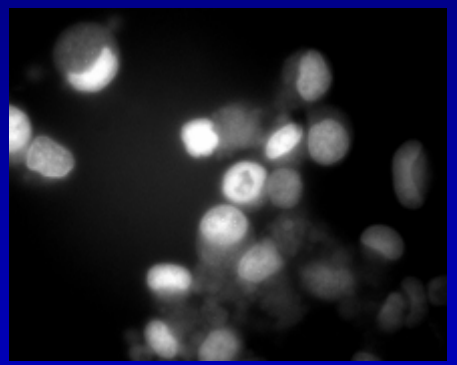
A. nuclei (DAPI)



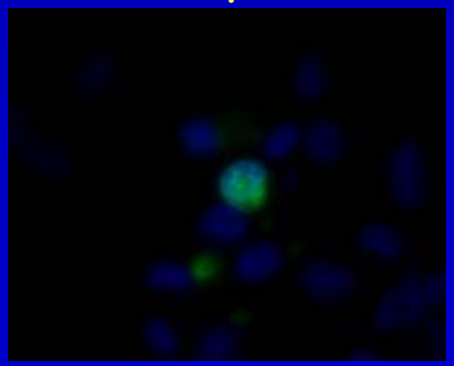
B. ΔRING-BARD-EGF



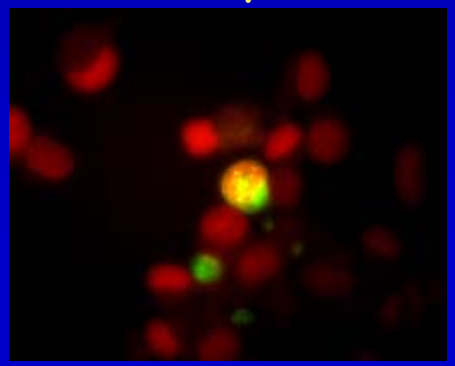
C. Anti-BARD1



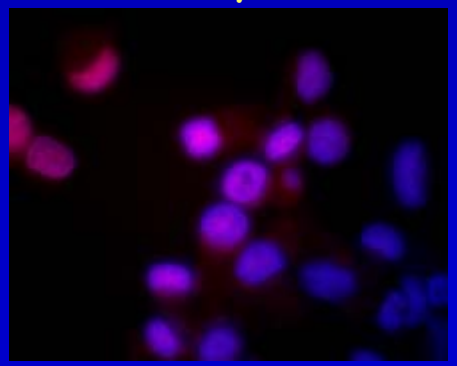
A+B overlay



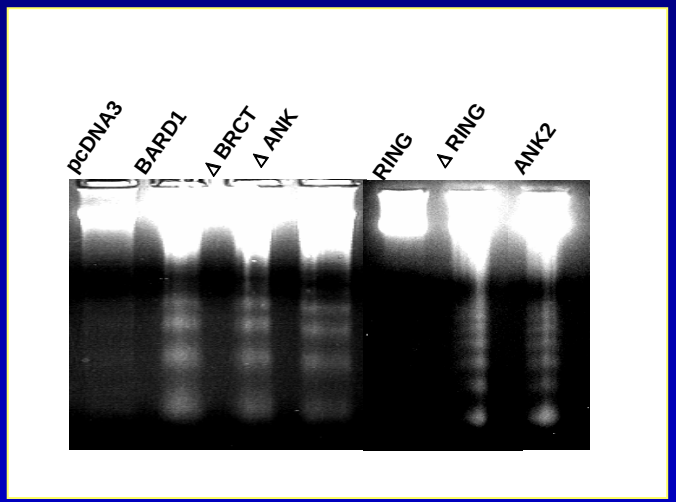
B+C overlay

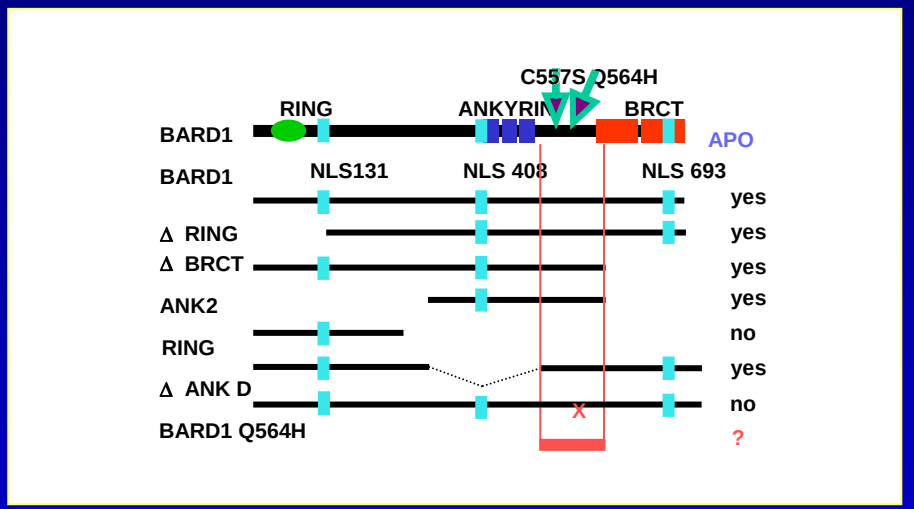


A+C overlay

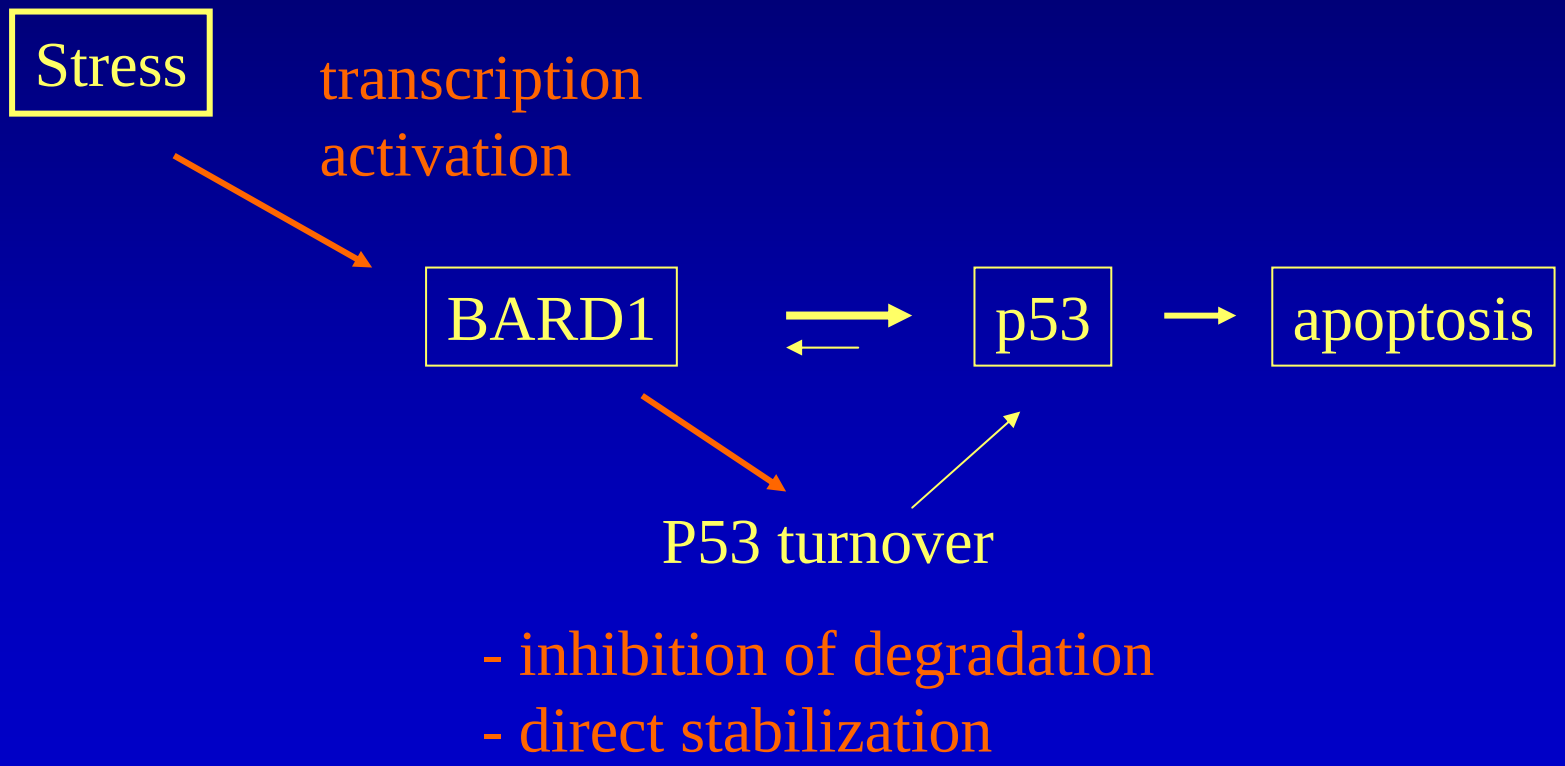


Mapping of the apoptotic region



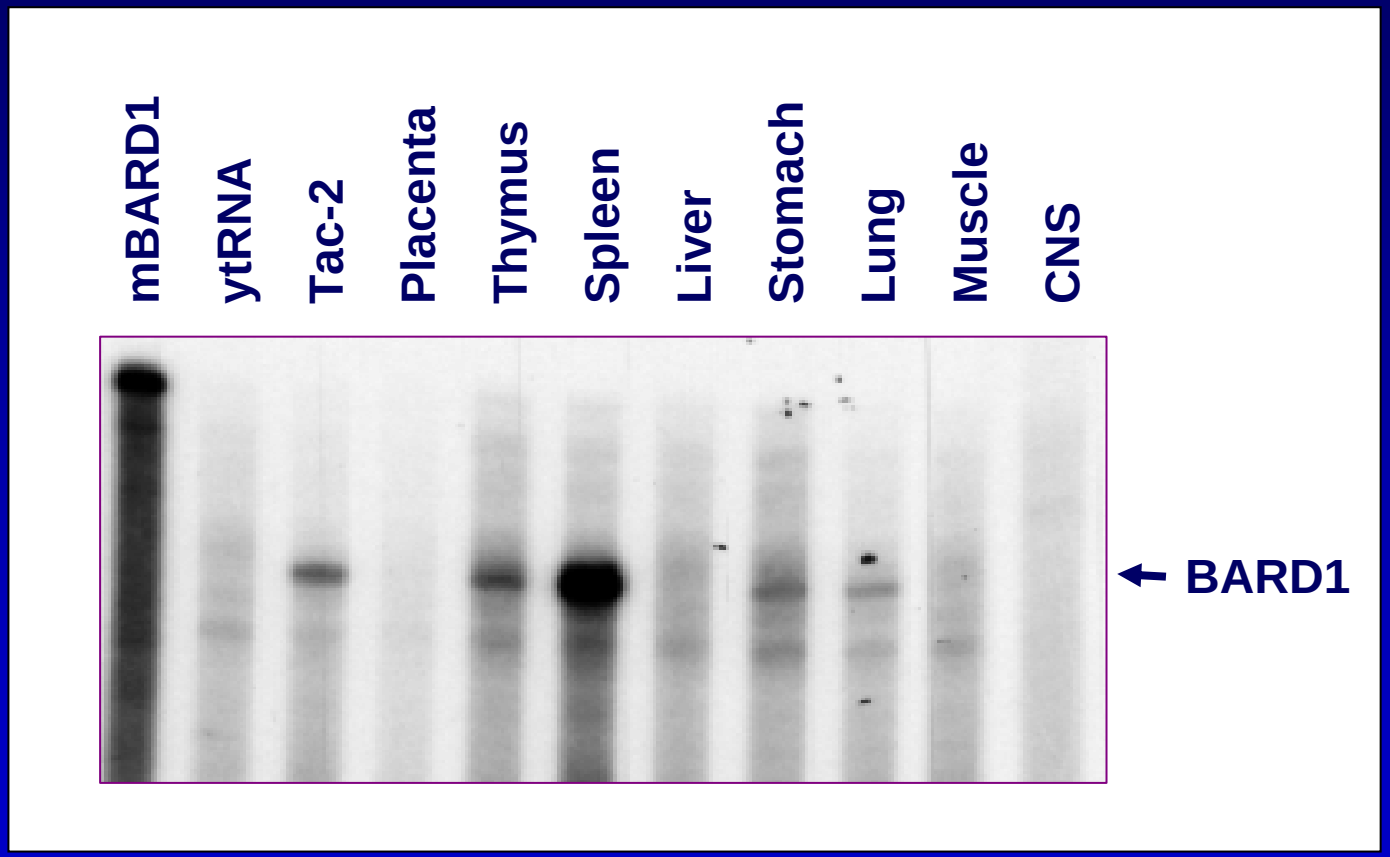


BARD1 acts in p53-mediated apoptotic pathway

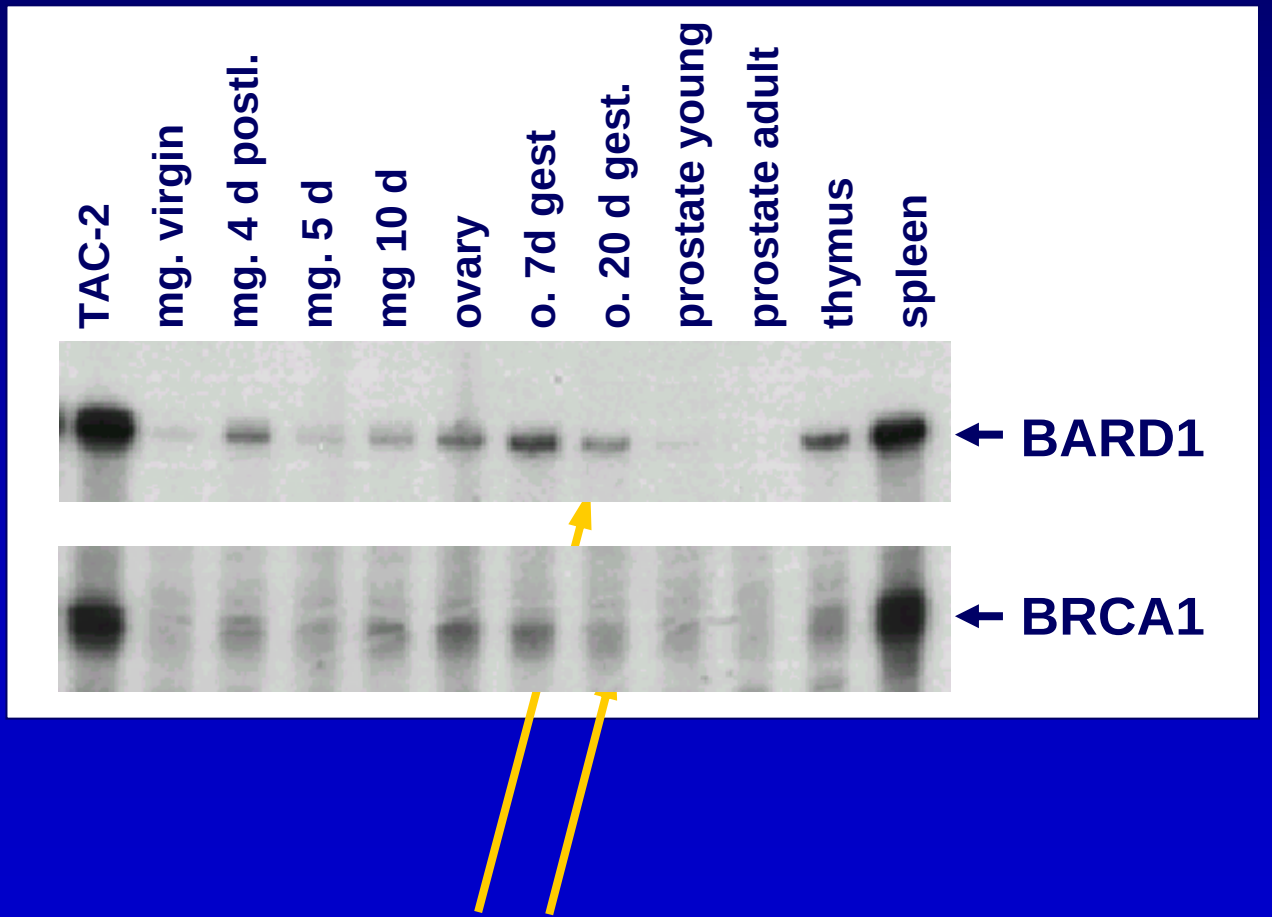


Non-correlated expression of BARD1 and BRCA1 - independent functions?

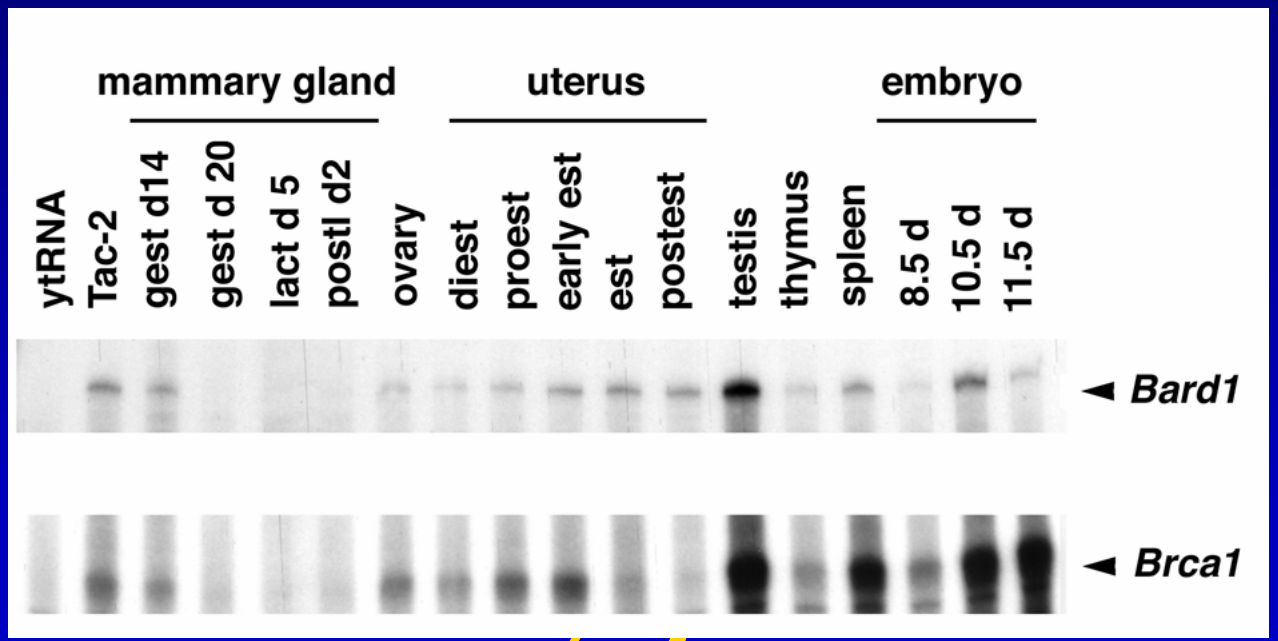
BARD1 is expressed in most rapidly dividing tissues



Correlated expression of BARD1 and BRCA1 in most tissues

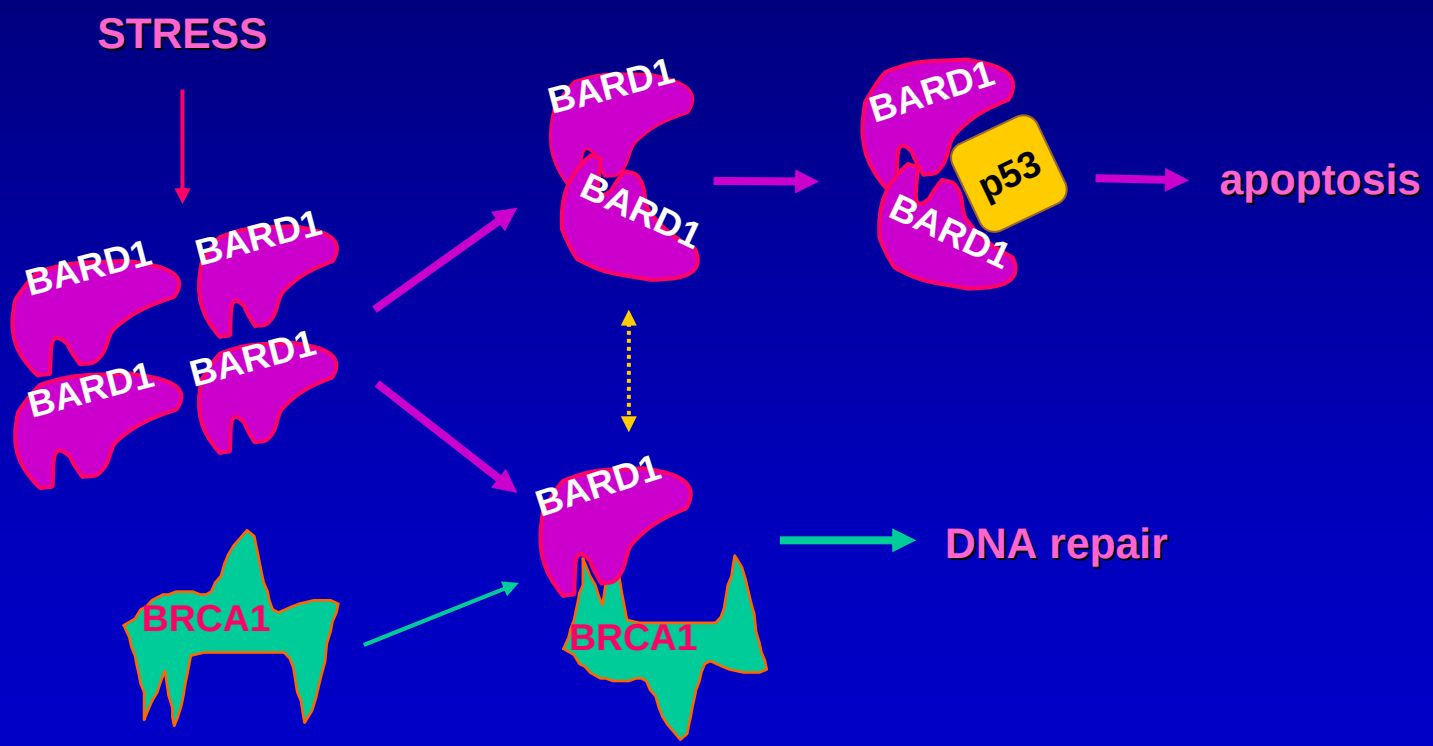


Non-correlated expression of BARD1 and BRCA1 in some tissues



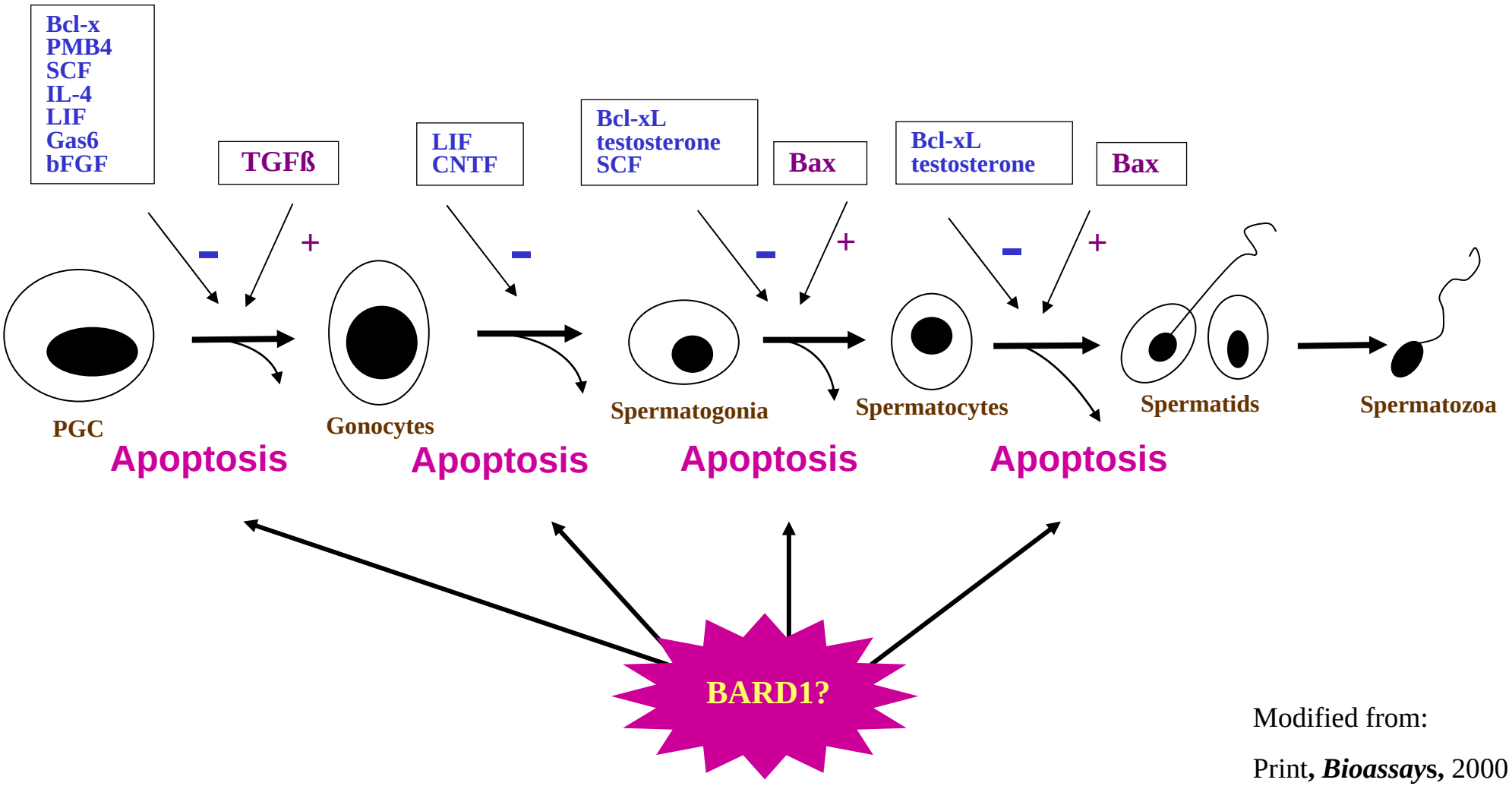
BARD1 expression in **absence** of BRCA1 is consistent with BARD1 but not BRCA1 mutations found in uterine tumors [Thai, Baer, *Hum Mol Gen* 1998].

Bimodal function of BARD1



Does bimodal function of BARD1 exist in vivo?

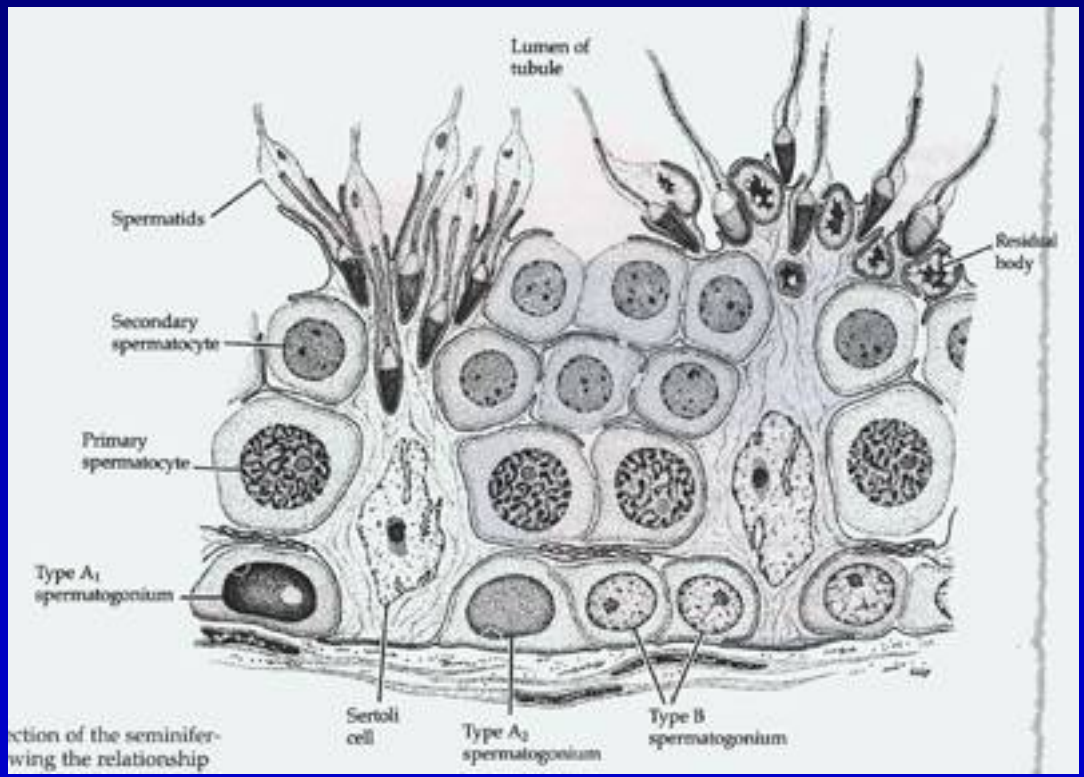
Apoptosis and (meiotic) repair during spermatogenesis



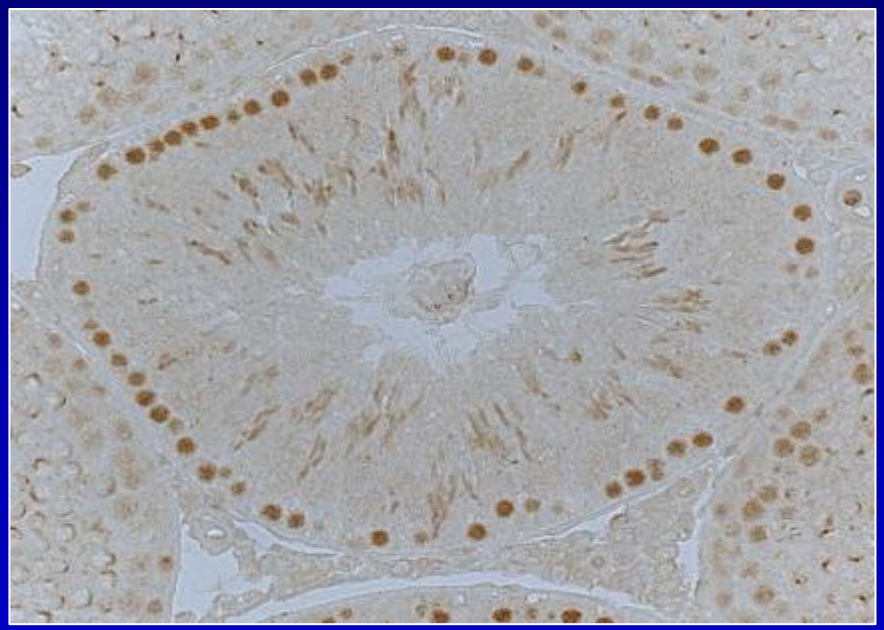
Modified from:

Print, ***Bioassays,*** 2000

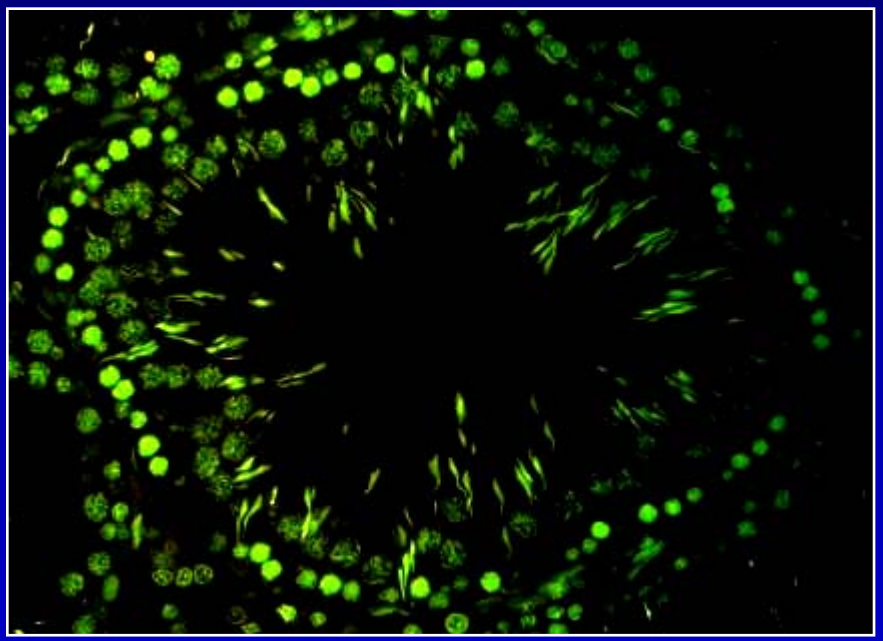
Schematic diagram of seminiferous tube



BARD1 expression is correlated with apoptosis

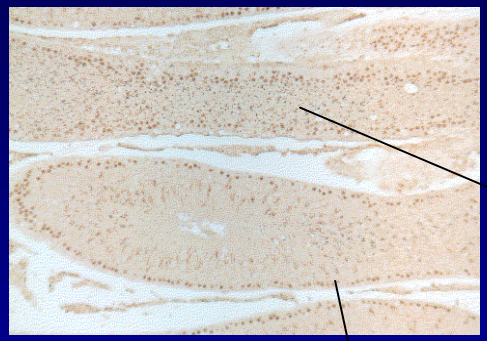


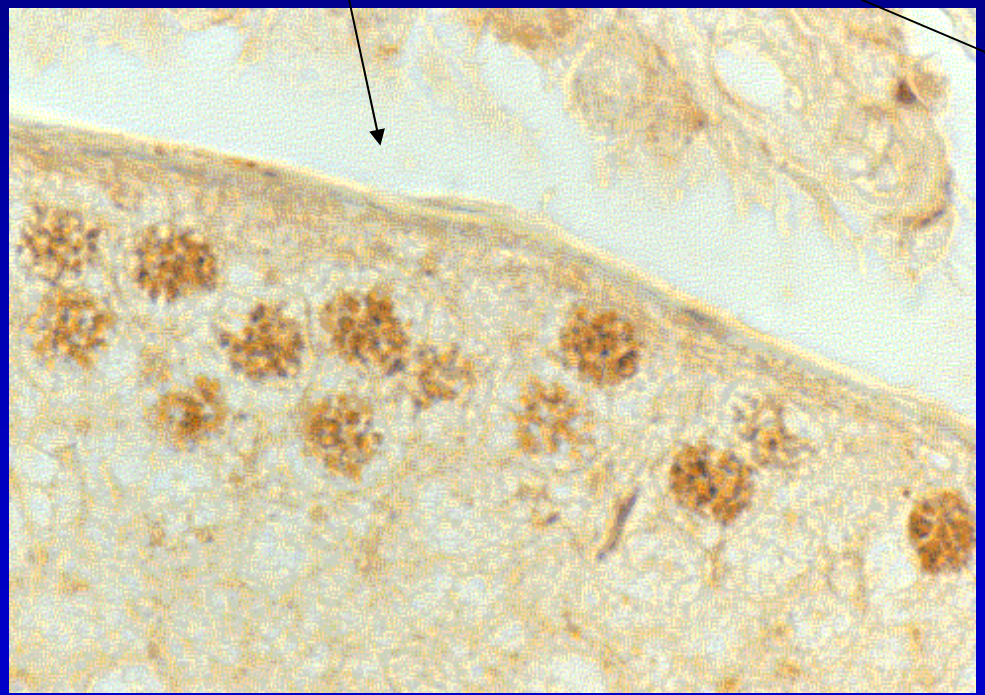
BARD1 N-19 antibody



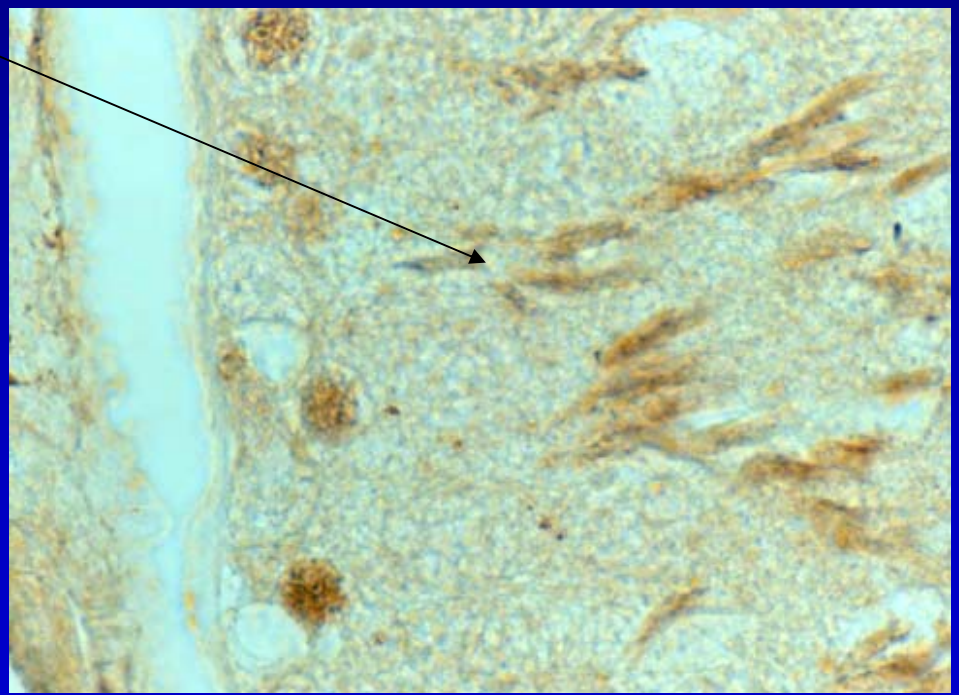
TUNEL

BARD1 immuno-localization in testis



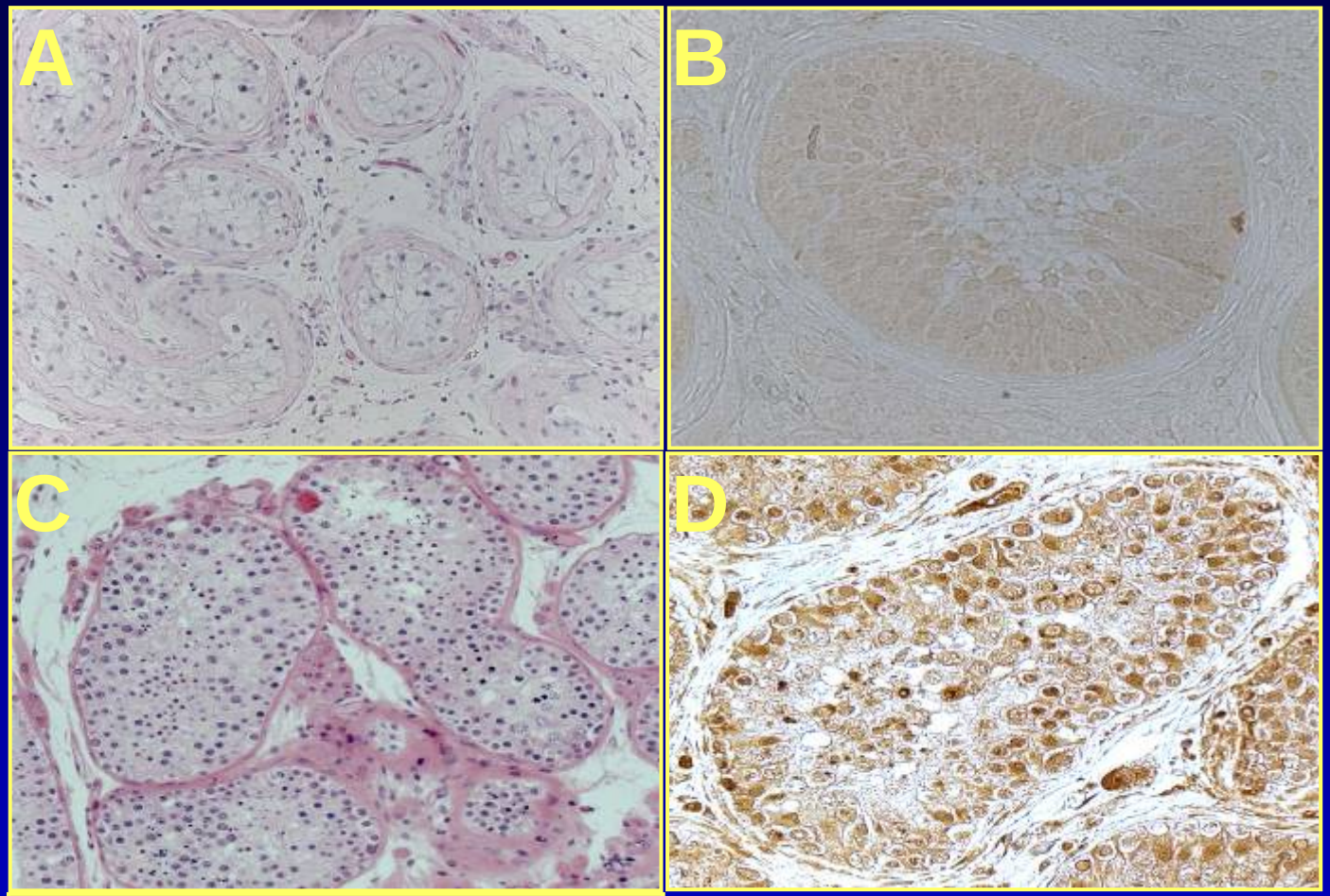


Premeiotic (N-19)

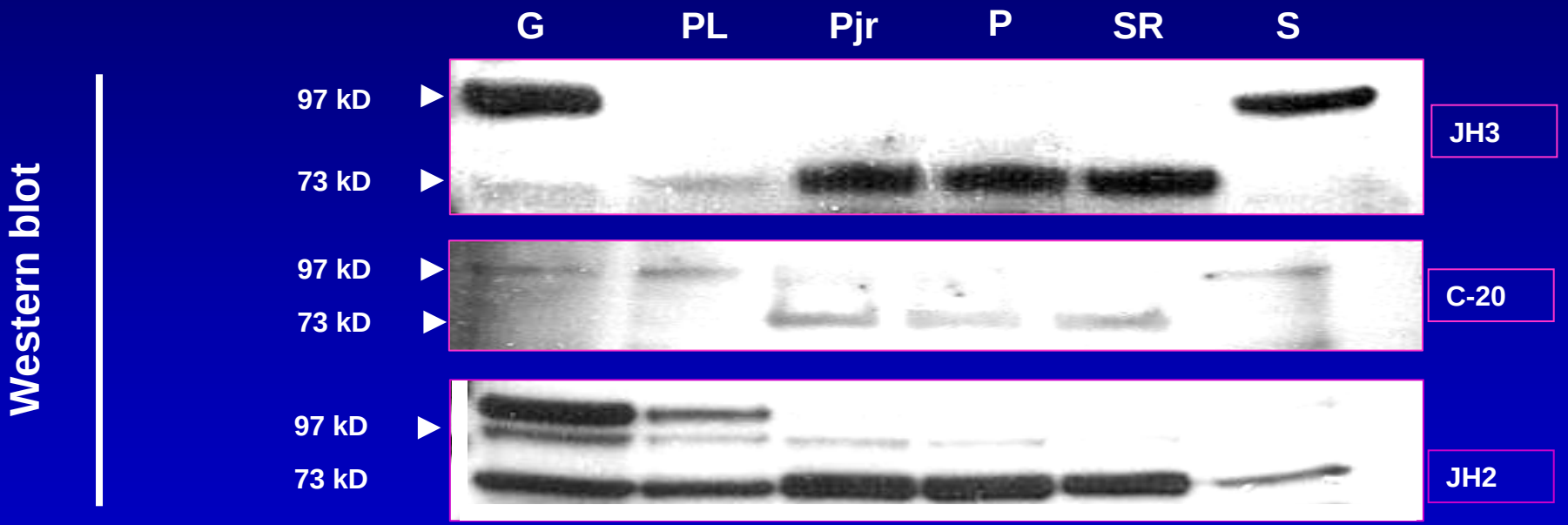


Postmeiotic (JH-3)

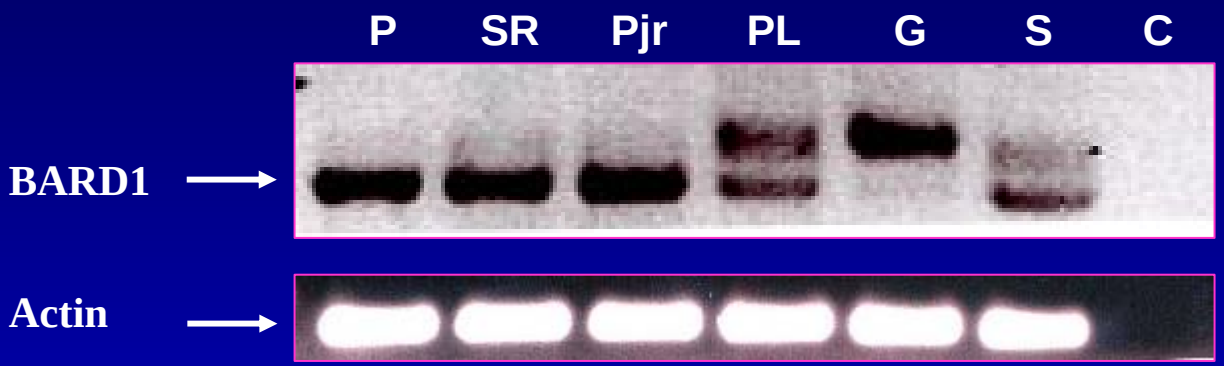
BARD1 absent in human Cryptorchidy



BARD1 isoforms in purified cells from testis



Differentially spliced BARD1 in testis



P: Pachytene spermatocyte (3 months old)

SR: Round spermatid (3 months old)

Pjr: Pachytene spermatocyte (23 days old)

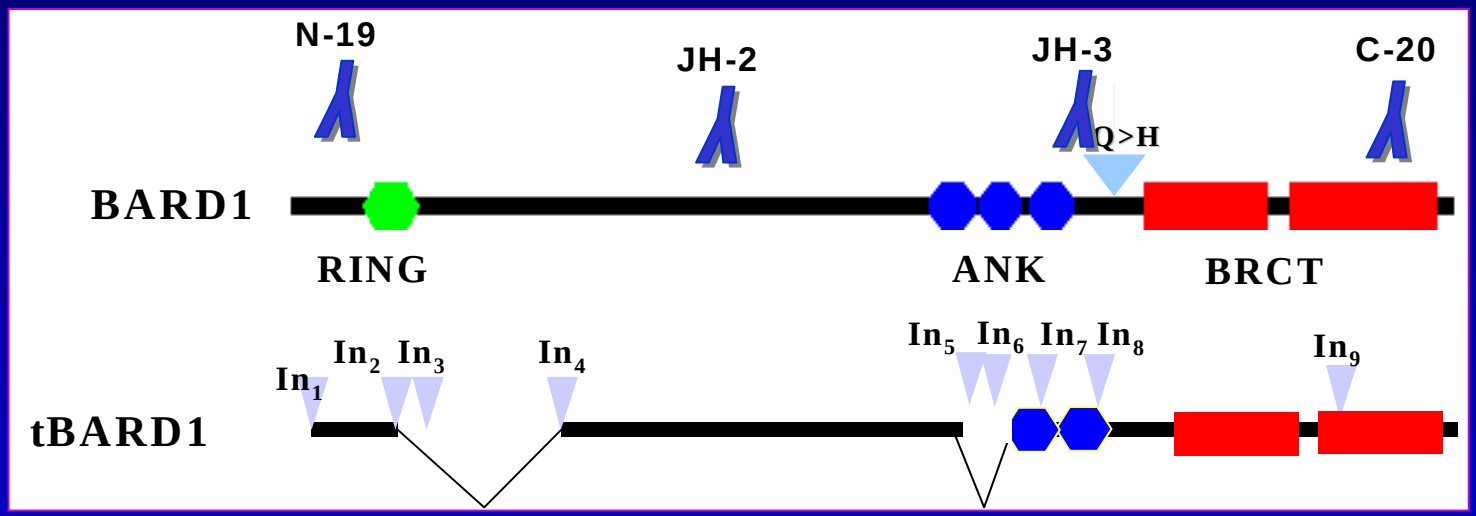
PL: Preleptotene spermatocyte (23 days old)

G: Spermatogonia (9 days)

S: Sertoli (3months old)

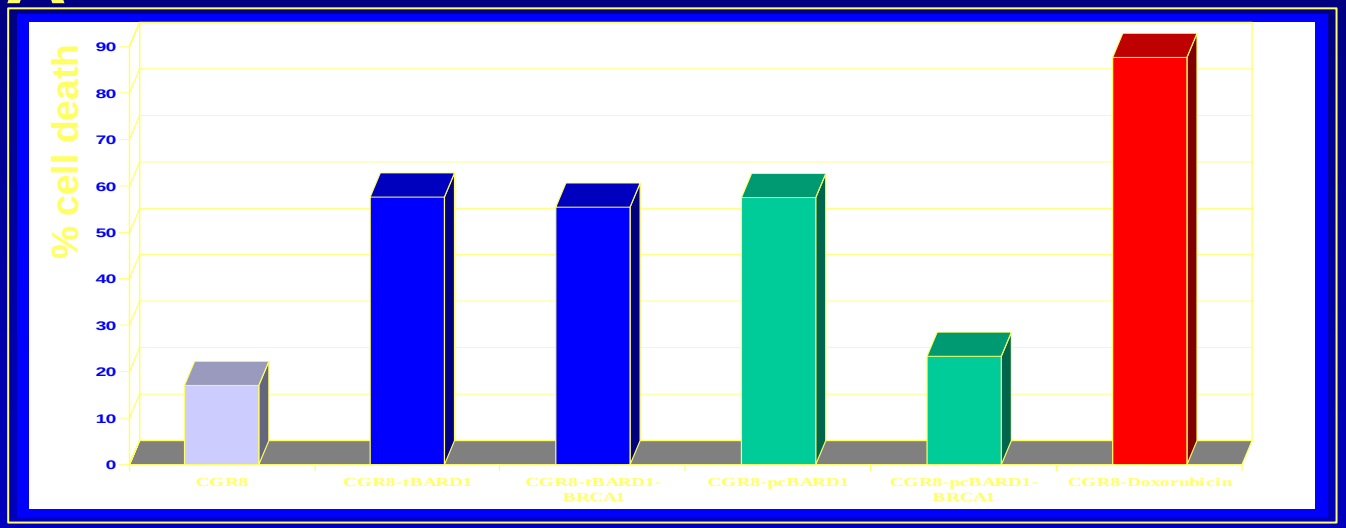
C: Control

tBARD1 is lacking the RING finger



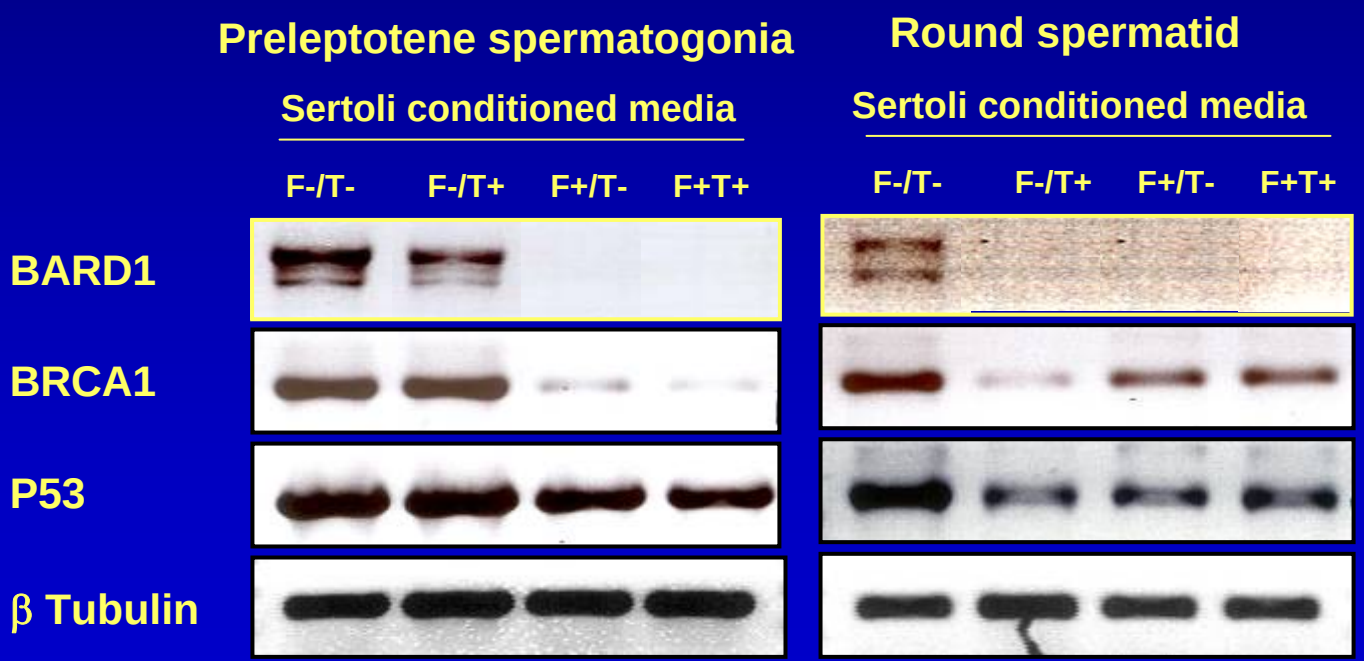
tBARD1 more efficient in apotosis induction

A

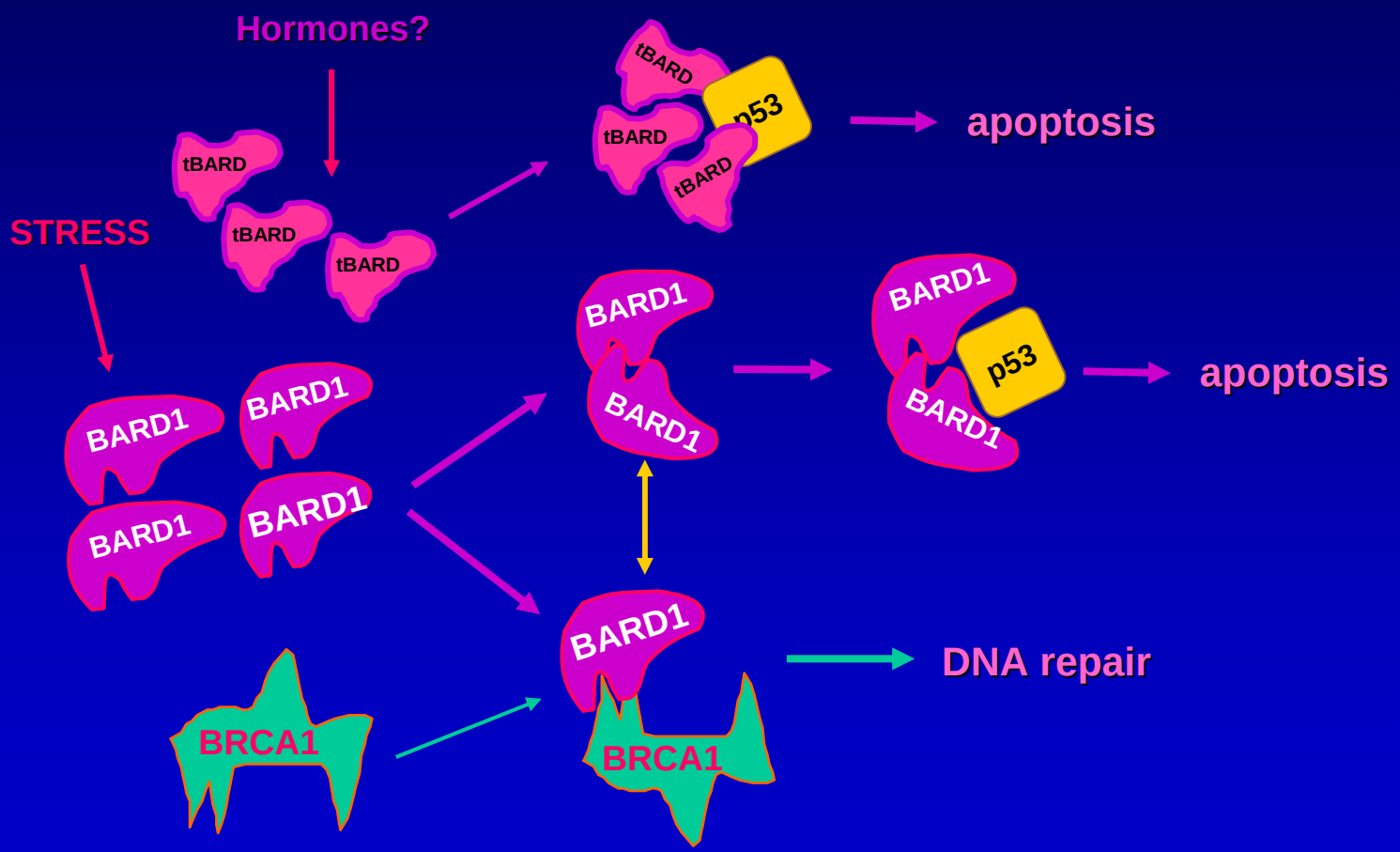


B

BARD1 expression is hormonally controlled



tBARD1 the superkiller



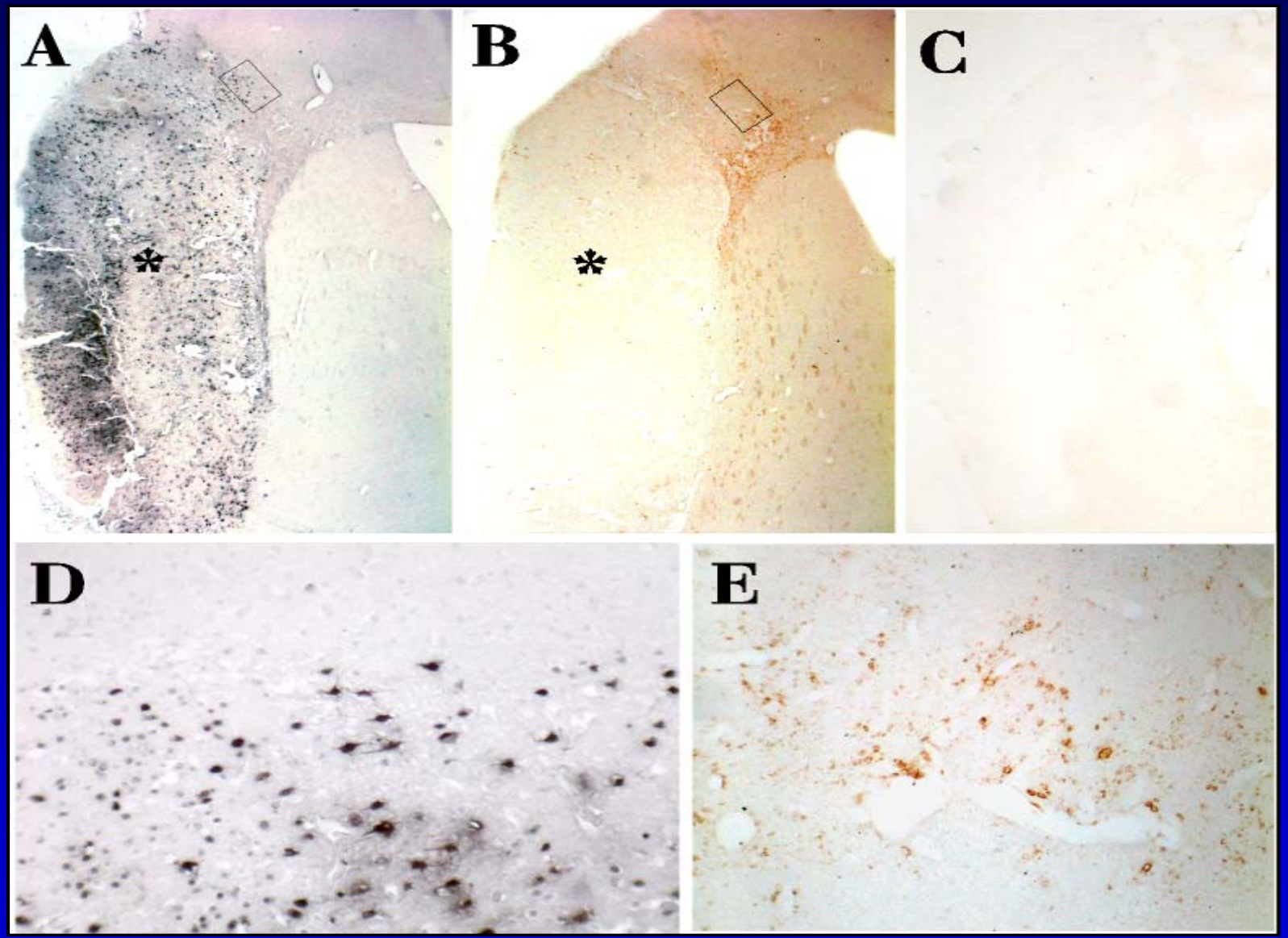
Two *in vivo* functions of BARD1

- *in complex with BRCA1* and other repair proteins
  - Cell cycle control and genomic stability
  - Repair functions

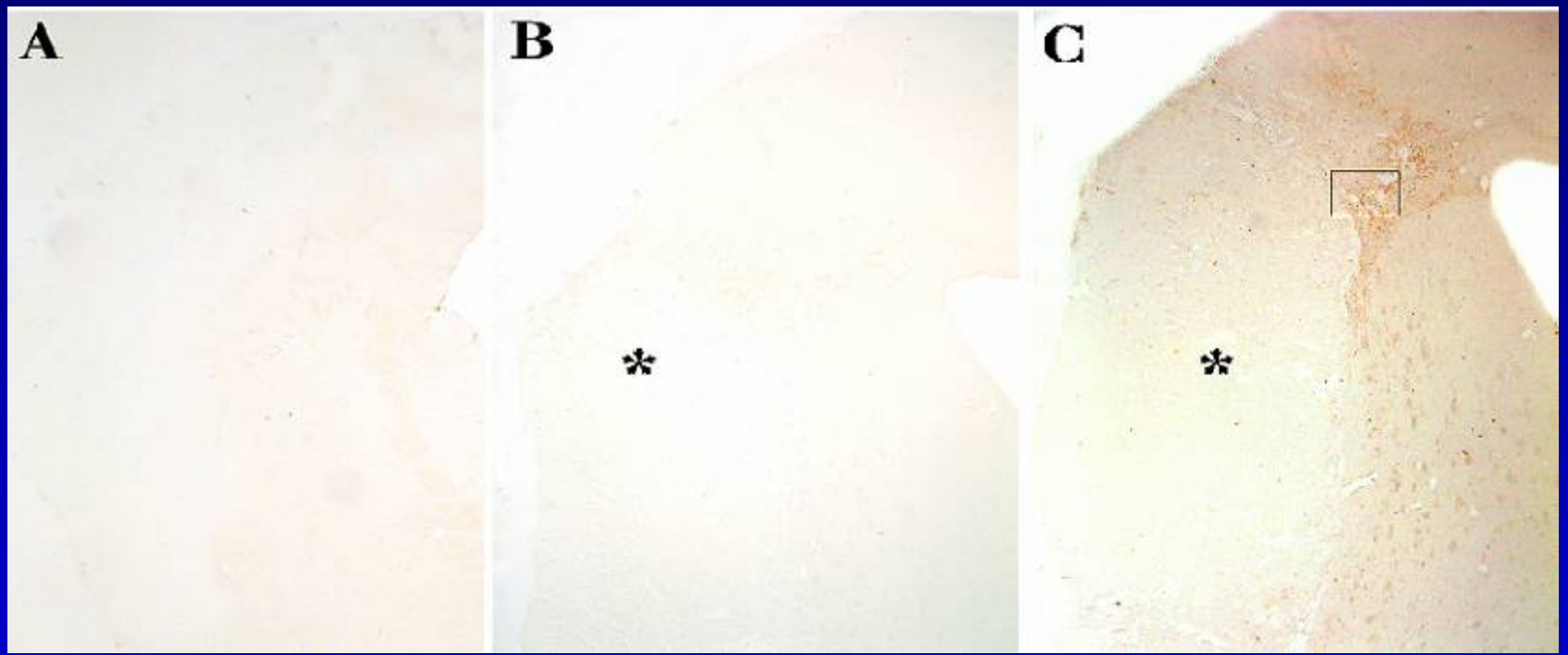
- *BRCA1-independent*: apoptotic functions
  - in response to stress
  - in maintenance of tissue homeostasis
  - in development (to be shown)

Does stress-dependent upregulation of BARD1 exist in vivo?

BARD1 expression in the brain after ischemia

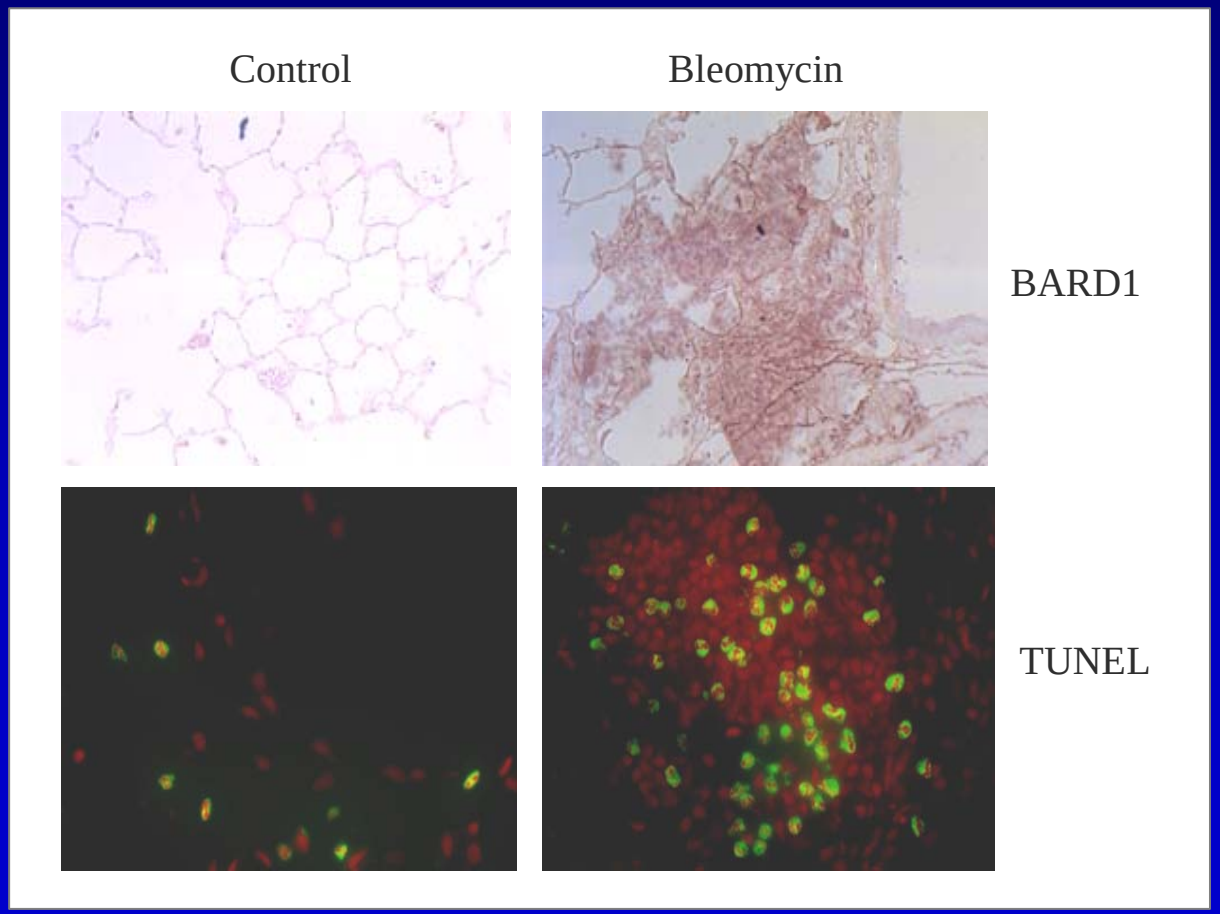


BARD1 but not BRCA1 is expressed in penumbra

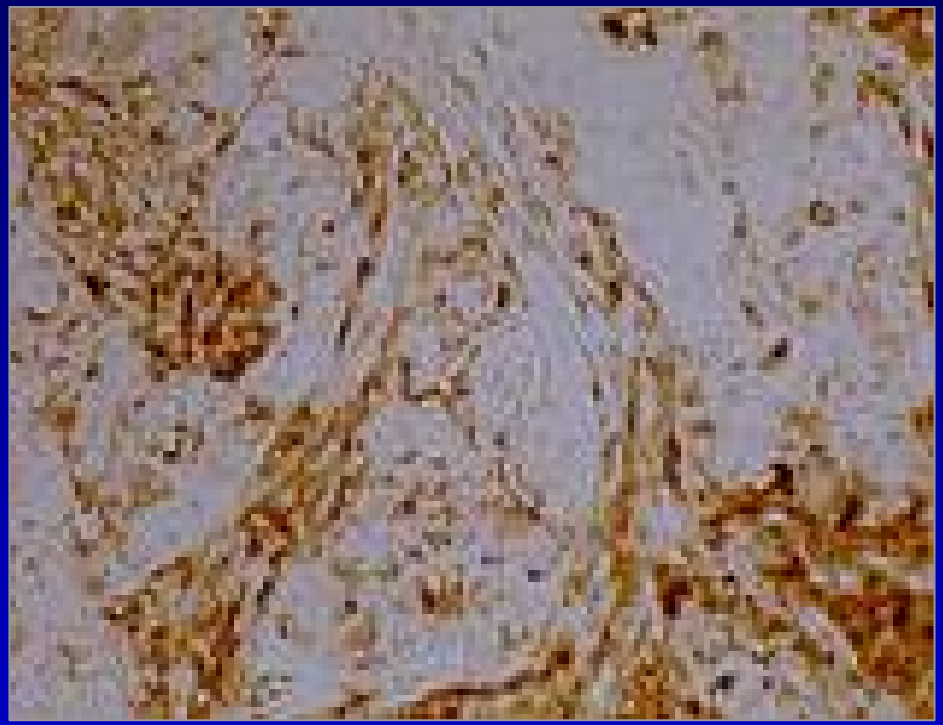


BARD1 induction in response to cellular stress

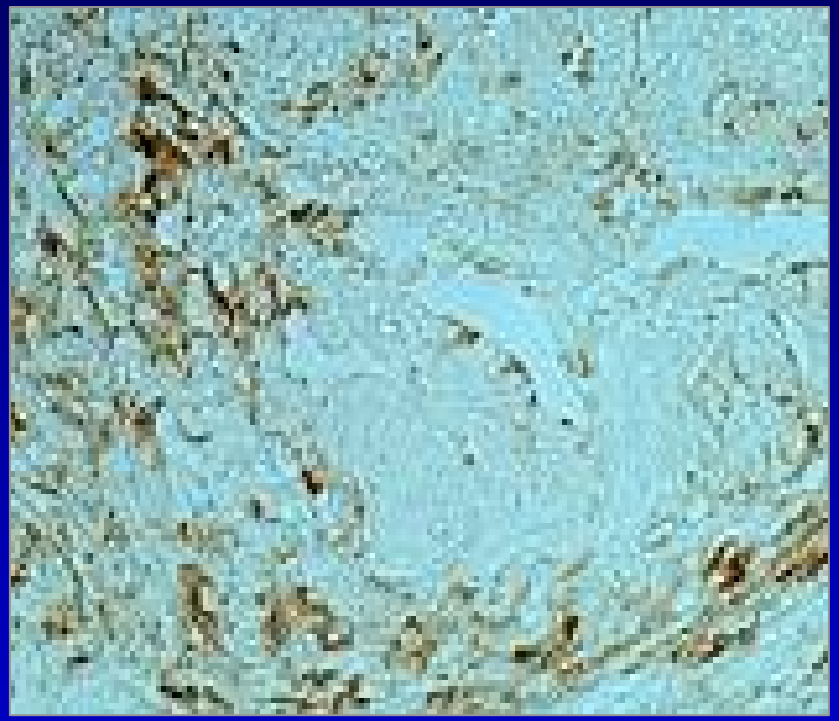
Upregulation of BARD1 associated with pre-malignant abnormal proliferation?



BARD1 upregulation in tumorigenesis?

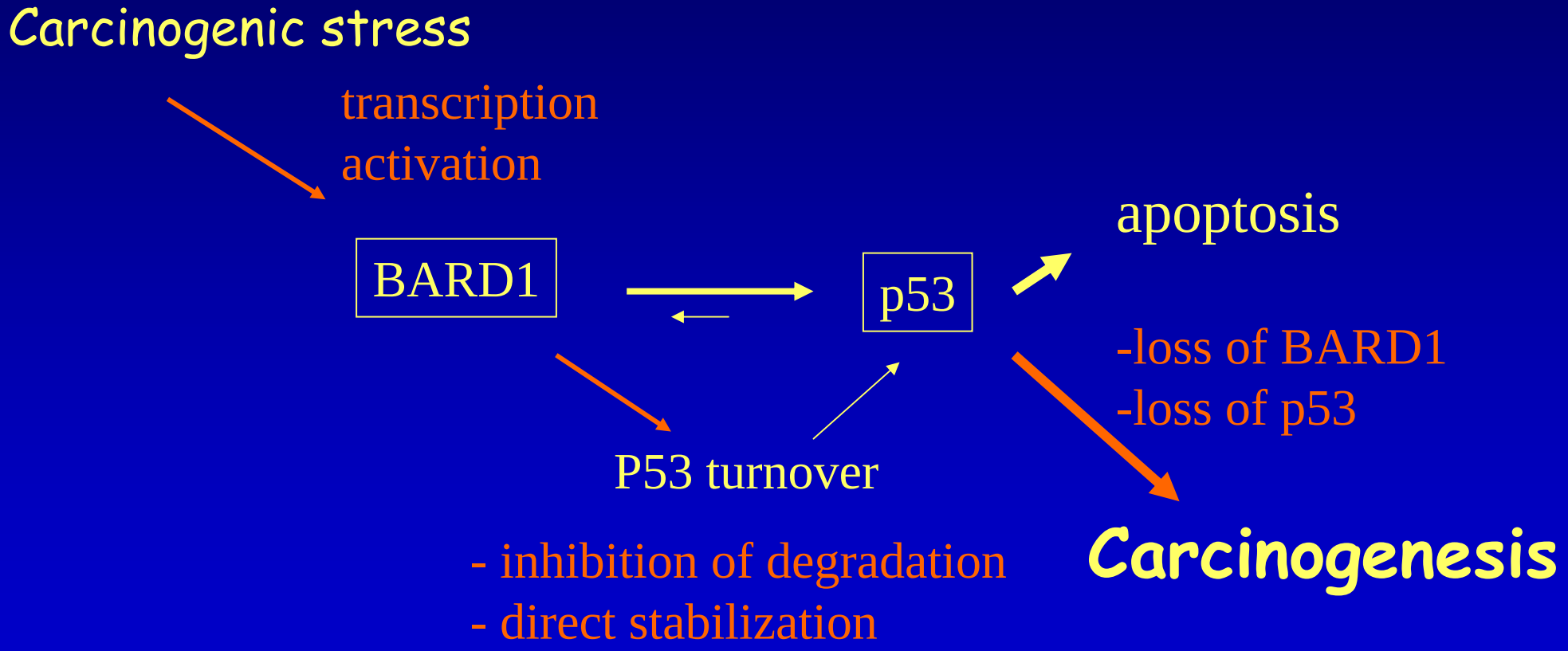


benign ovarian tumor

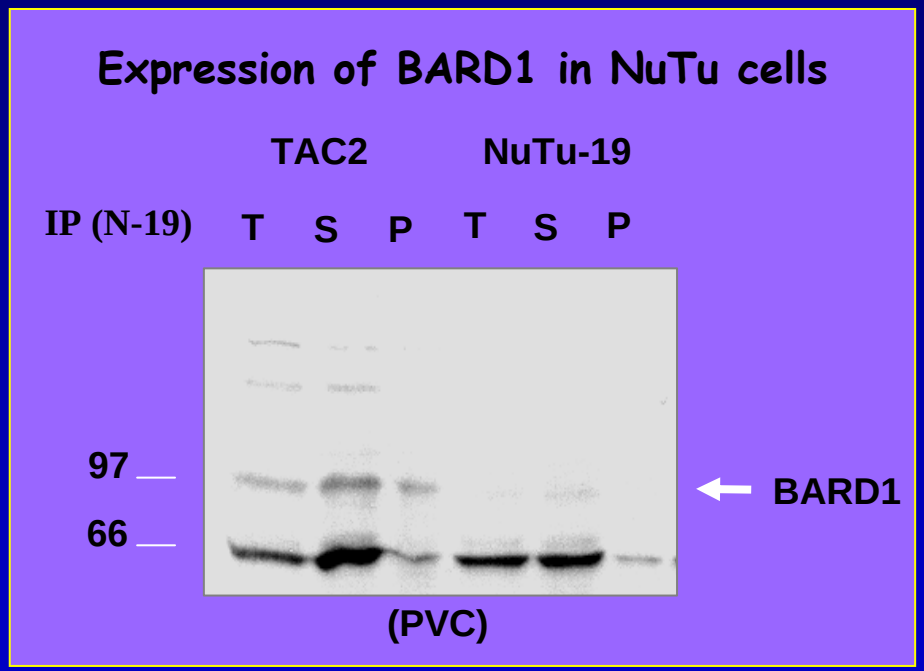


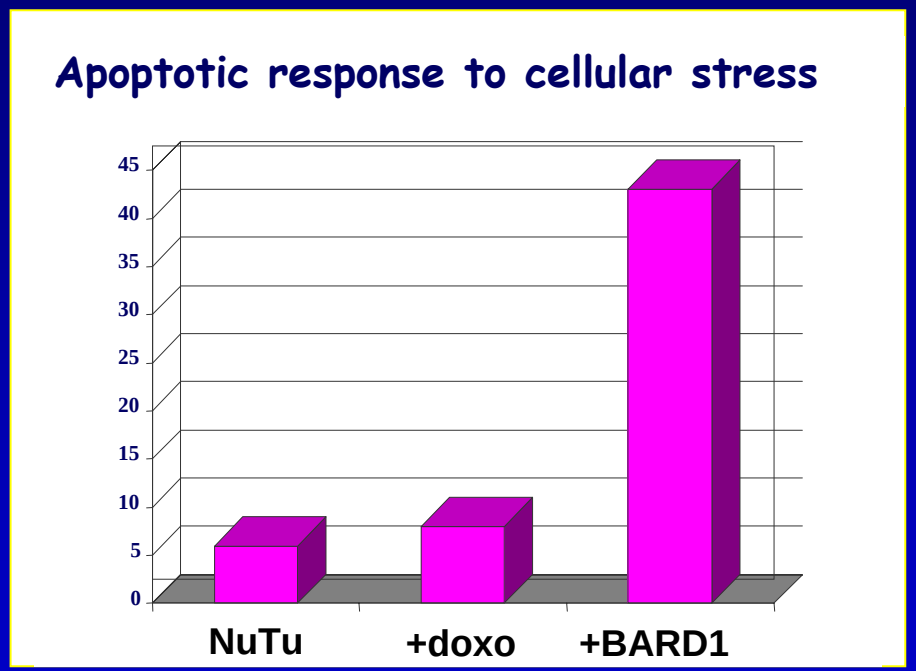
malignant ovarian tumor

Lack of BARD1 permissive of tumorigenesis

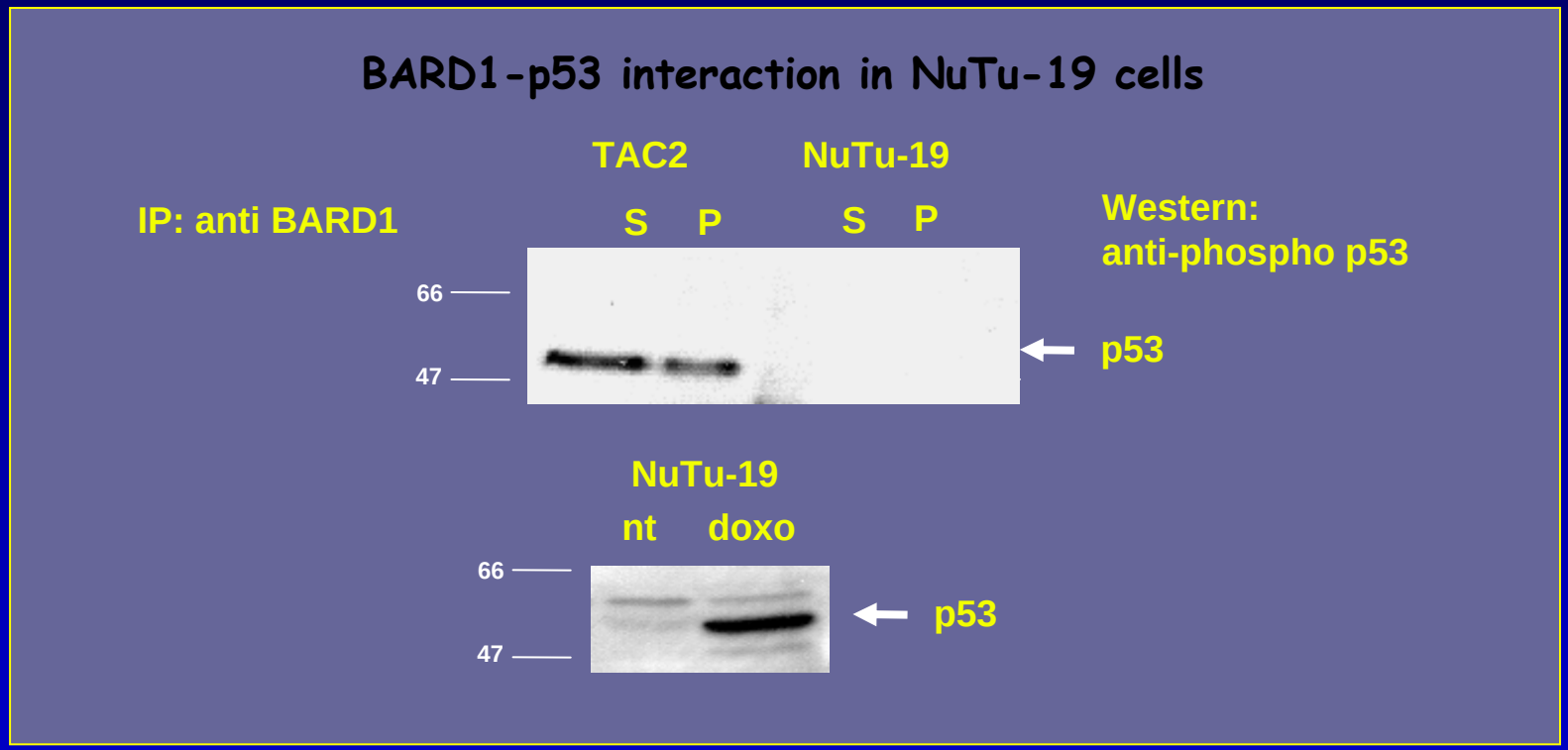


Loss of BARD1 function in malignant cancer cells
NuTu-19





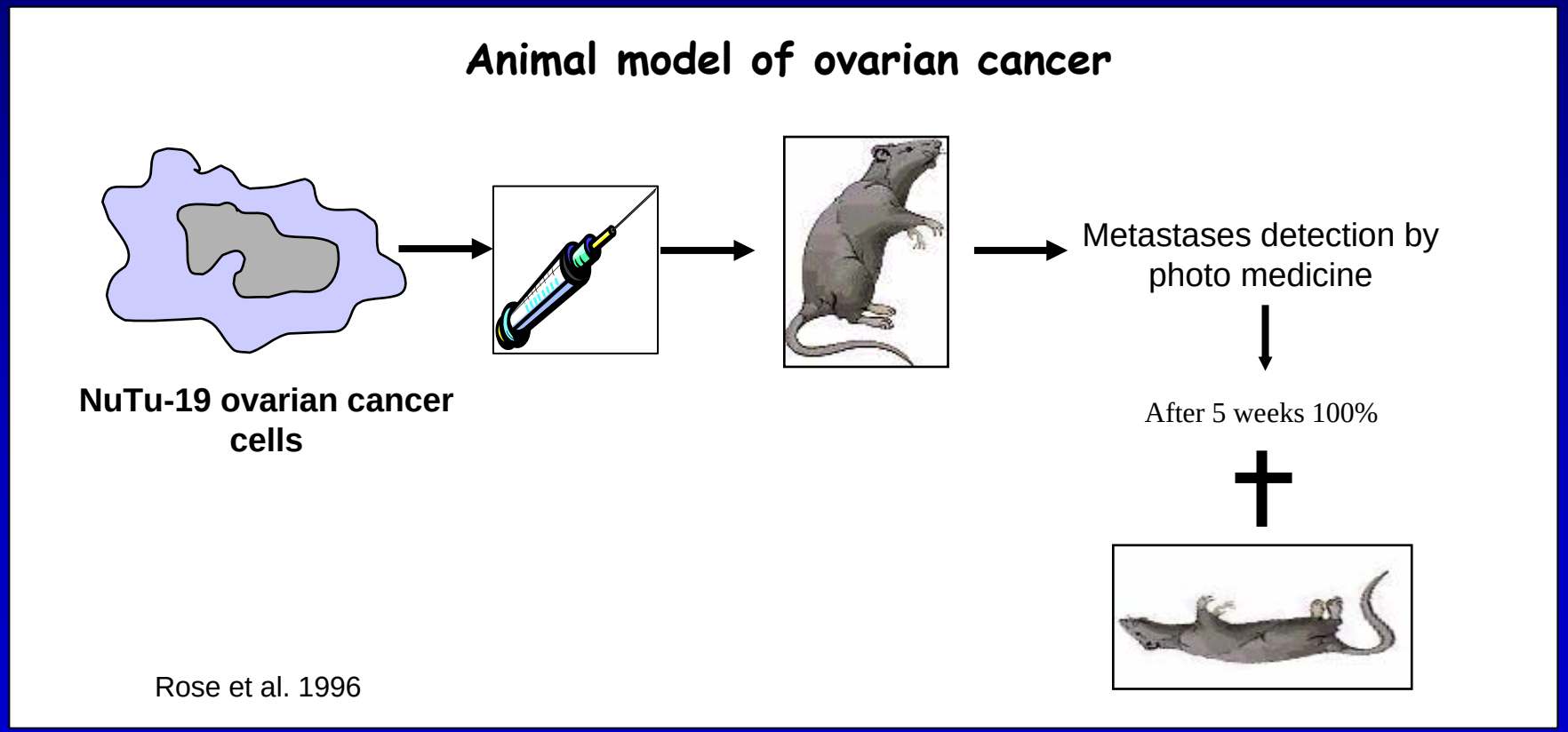
Defect of BARD1 pathway in NuTu-19 cells



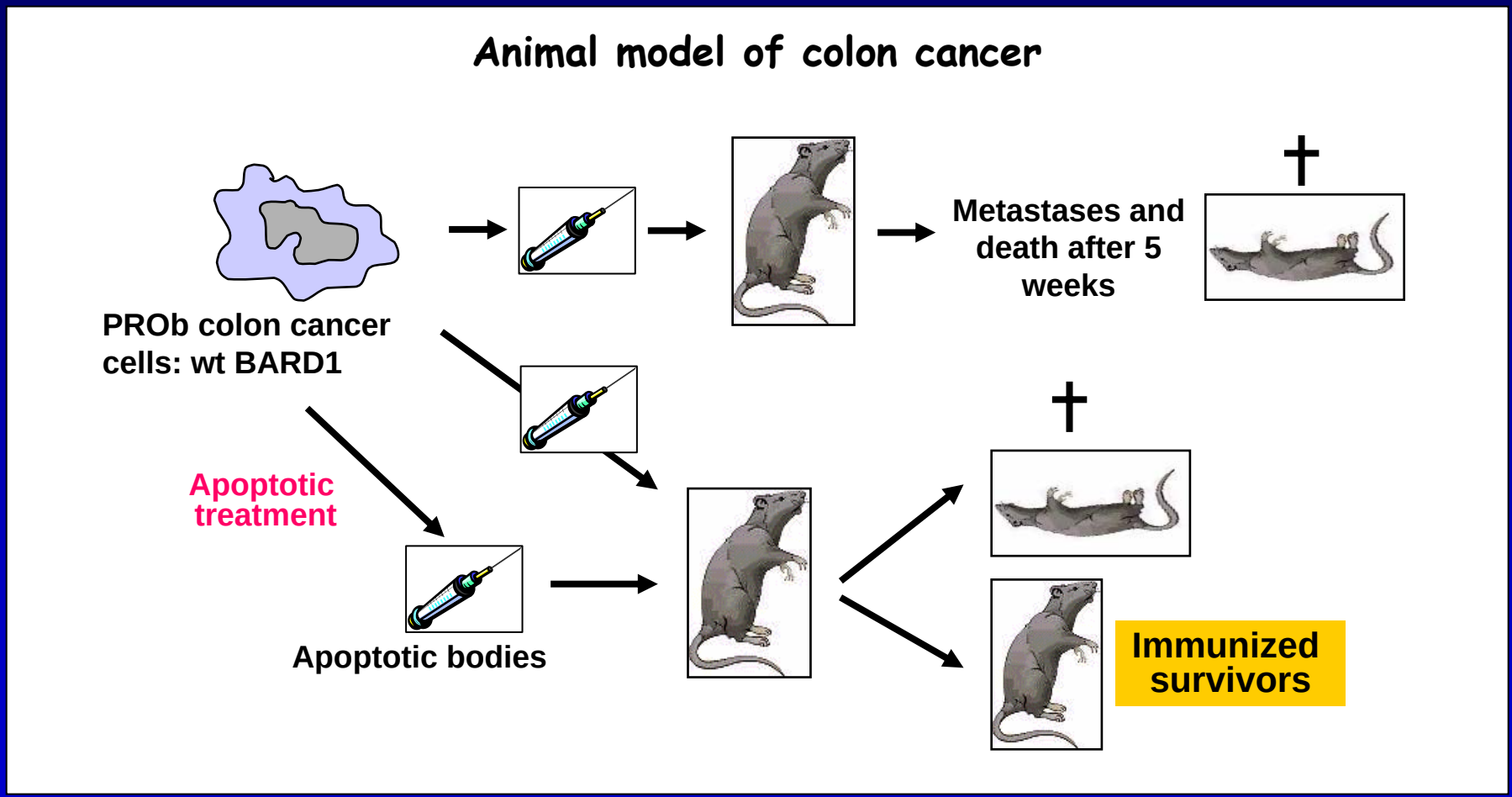
Conclusion: NuTu-19 cells express truncated form of BARD1
phosph p53 (ser37) is absent in NuTu-19 cells
p53 is present in NuTu-19 and co-IP with BARD1

BARD1 a tumor antigen

Animal model to test BARD1 function in tumorigenesis



Search for tumor antigens



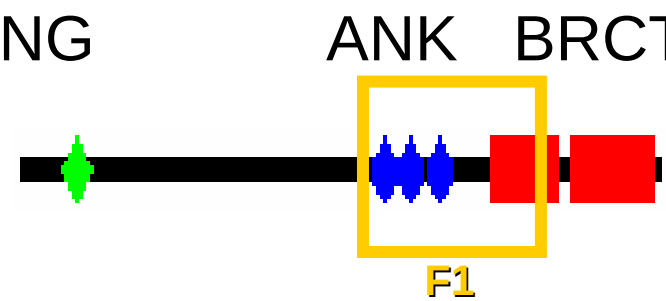
Identification of BARD1 as tumor antigen

Immunized survivors

Serum

Identification of *p67* on Western blots

Screening of rat cDNA expression libraries

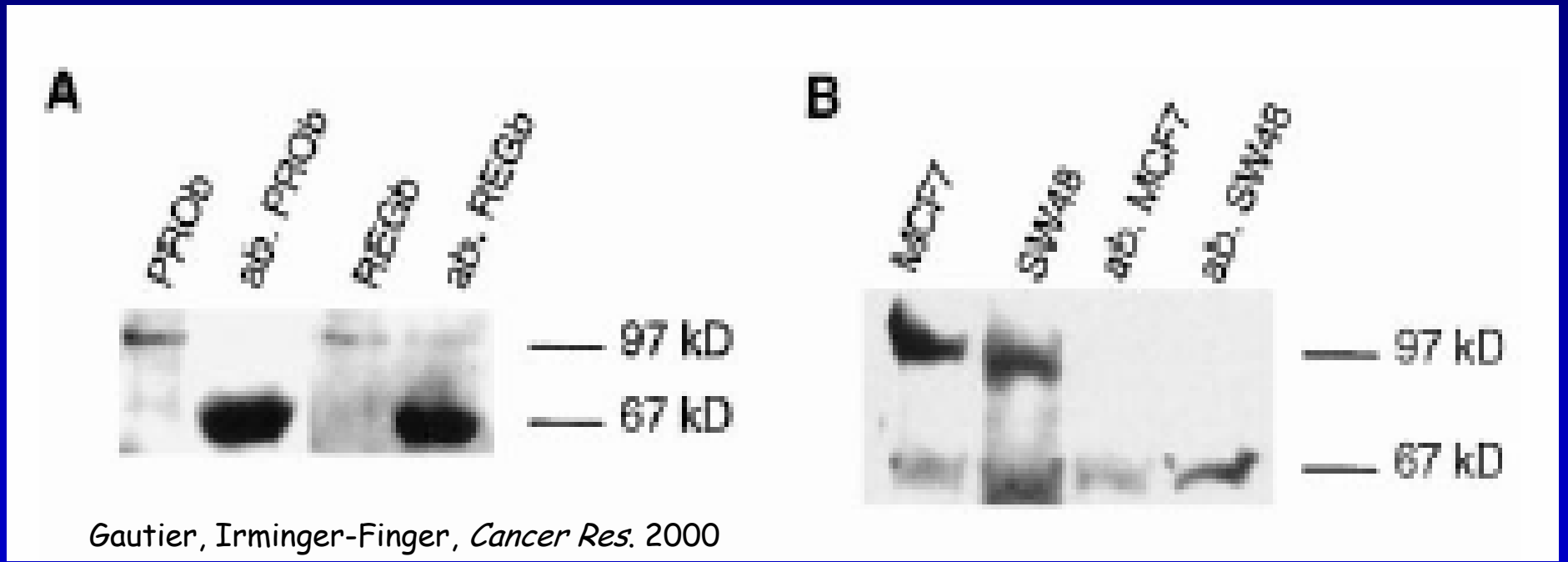


Identification of cDNA encoding C-terminal portion of BARD1

Vaccination with F1 renders rats partially immune to tumorgenesis

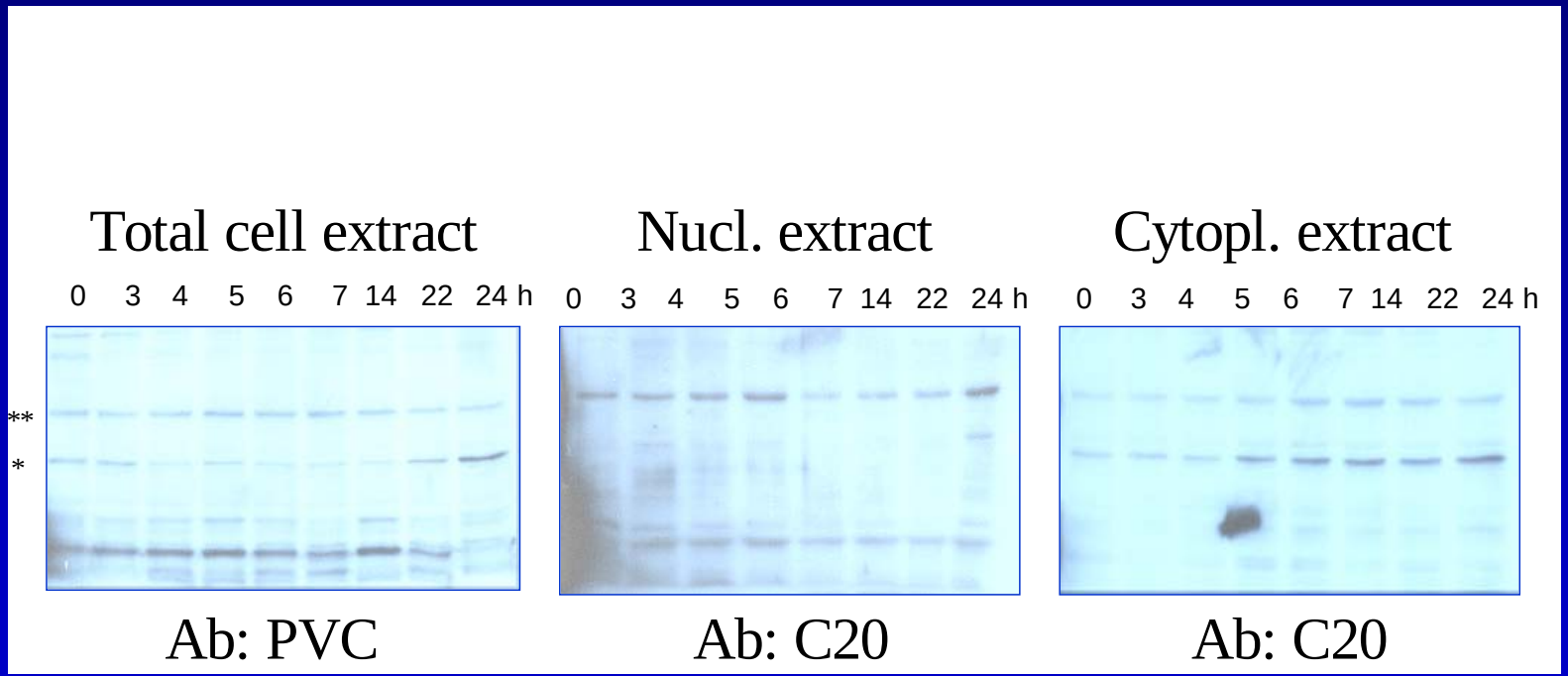
Gautier, Irminger-Finger, *Cancer Res.* 2000

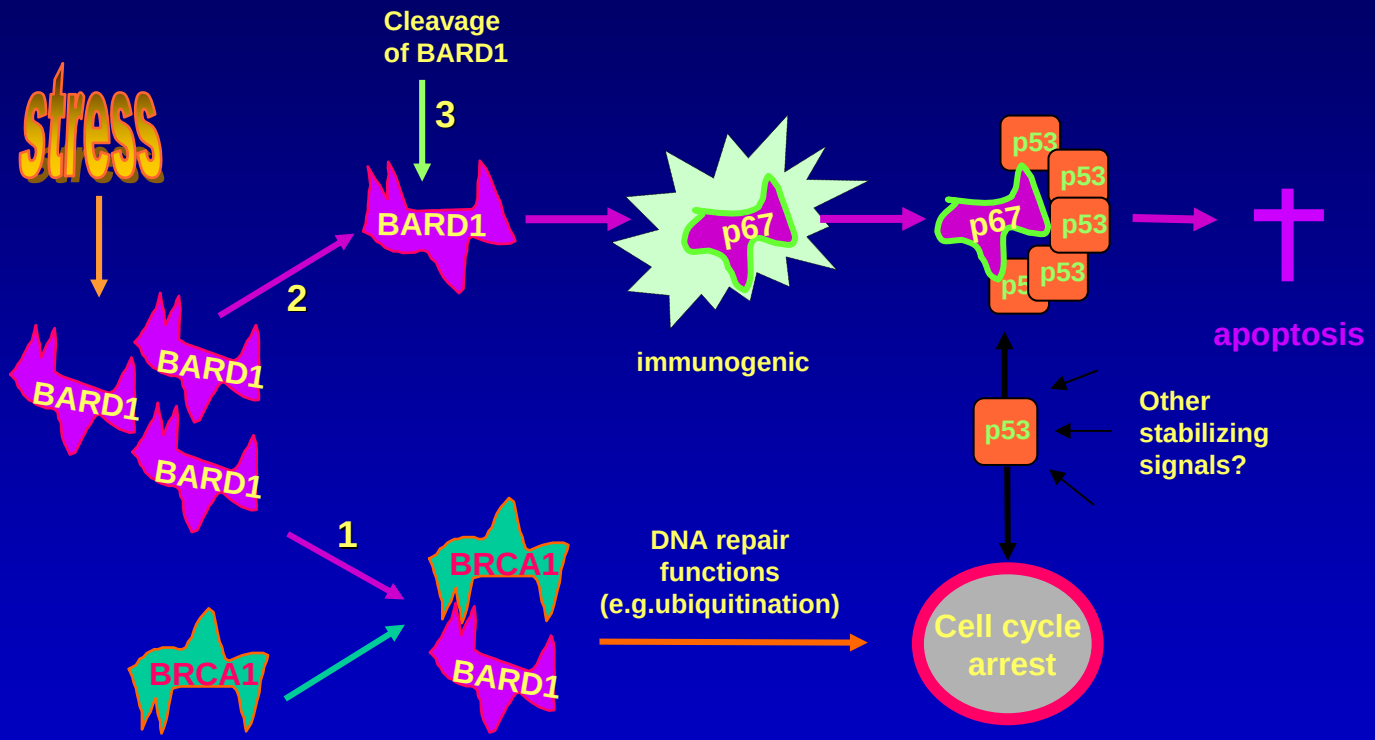
BARD1 upregulation and cleavage associated with apoptosis



Gautier, Irminger-Finger, *Cancer Res.* 2000

BARD1 is translocated to the cytoplasm during apoptosis

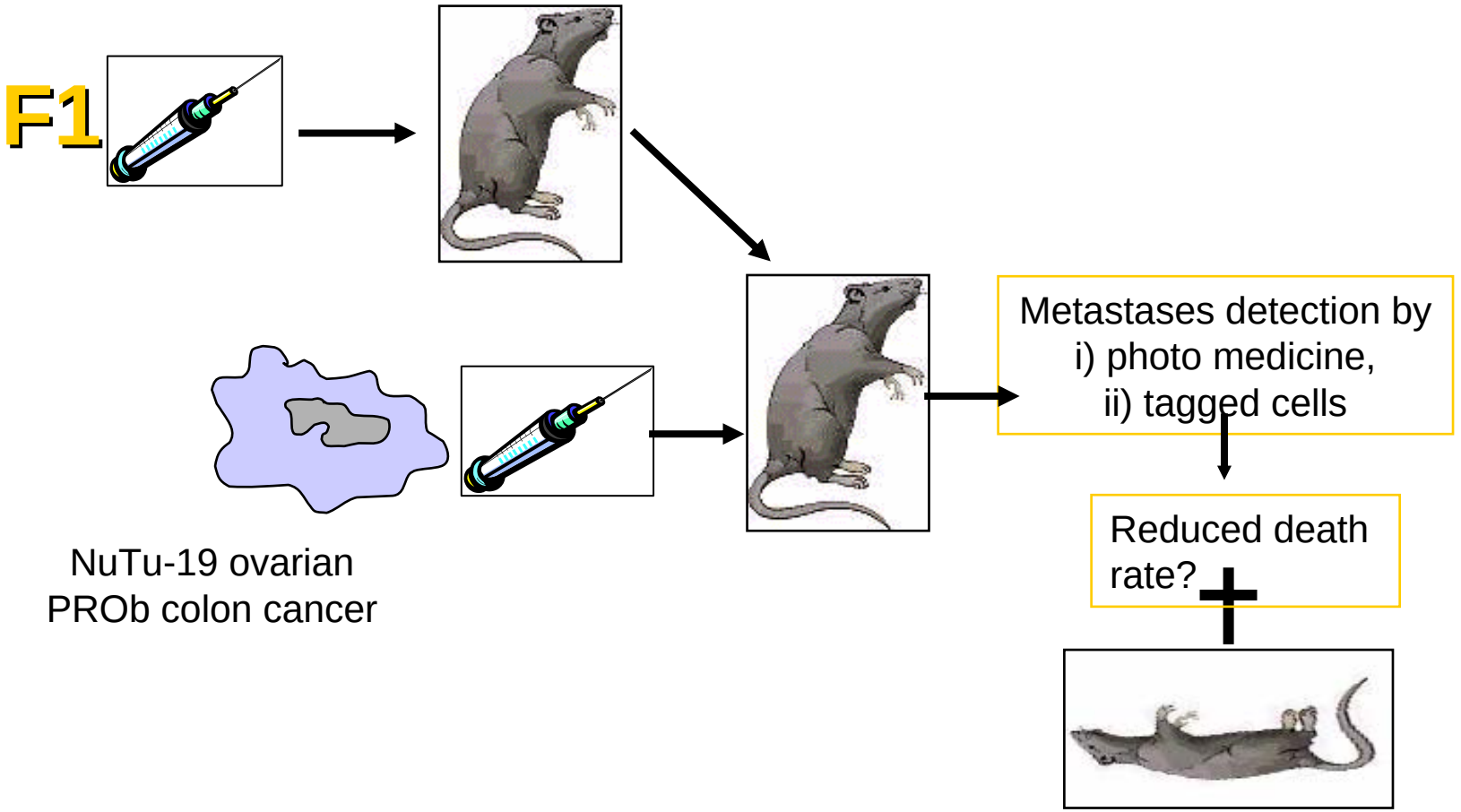



Cleavage
of BARD1
3
stress
BARD1
2
BARD1
BARD1
BARD1
p67
immunogenic
p53
p53
p67
p53
p53
p5
apoptosis
p53
Other
stabilizing
signals?
1
BRCA1
BRCA1
BARD1
DNA repair
functions
(e.g.ubiquitination)
Cell cycle
arrest

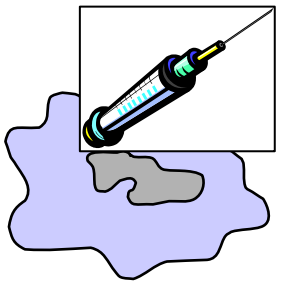
Applications

Cancer vaccine based on p67BARD1
Gene therapy: killing of unwanted cells
Antisense: promote cell survival

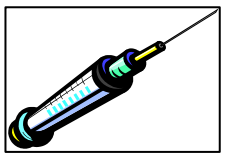
Application: Immuntherapy



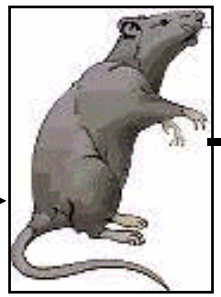
Application: Gene therapy



NuTu-19 ovarian or
PRObe colon cancer



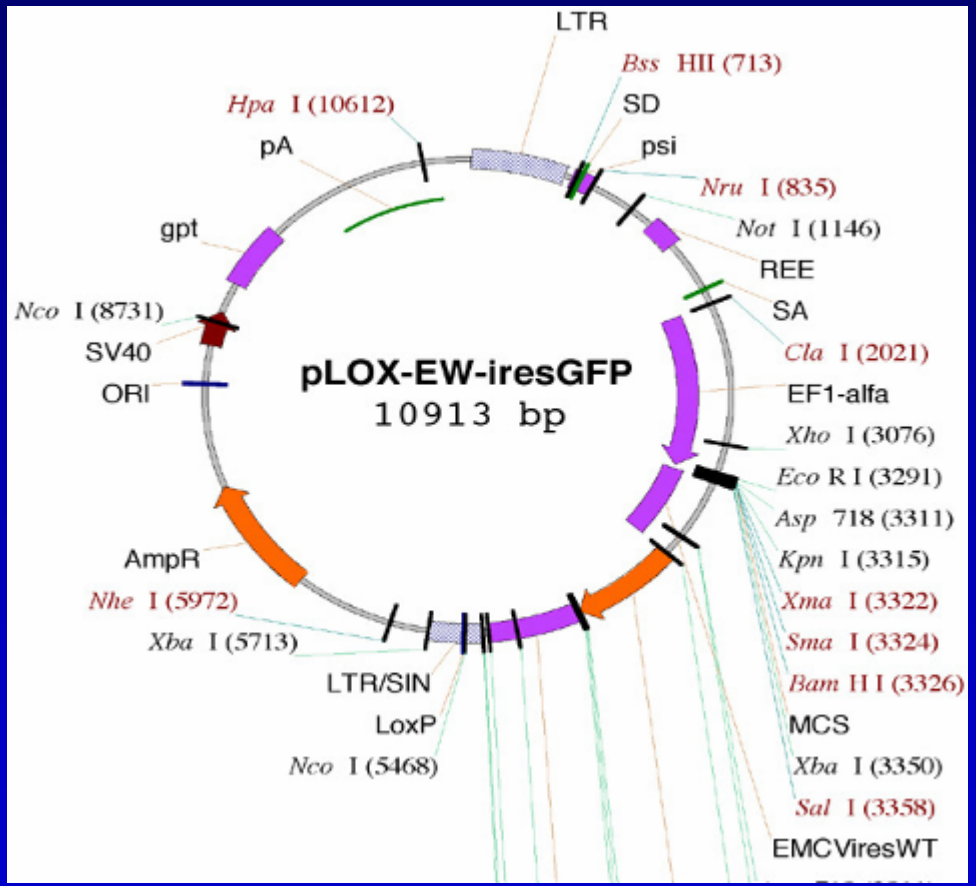
Viral delivery of BARD1
Adeno or retroviral vector



Metastases detection by
i) photo medicine,
ii) tagged cells

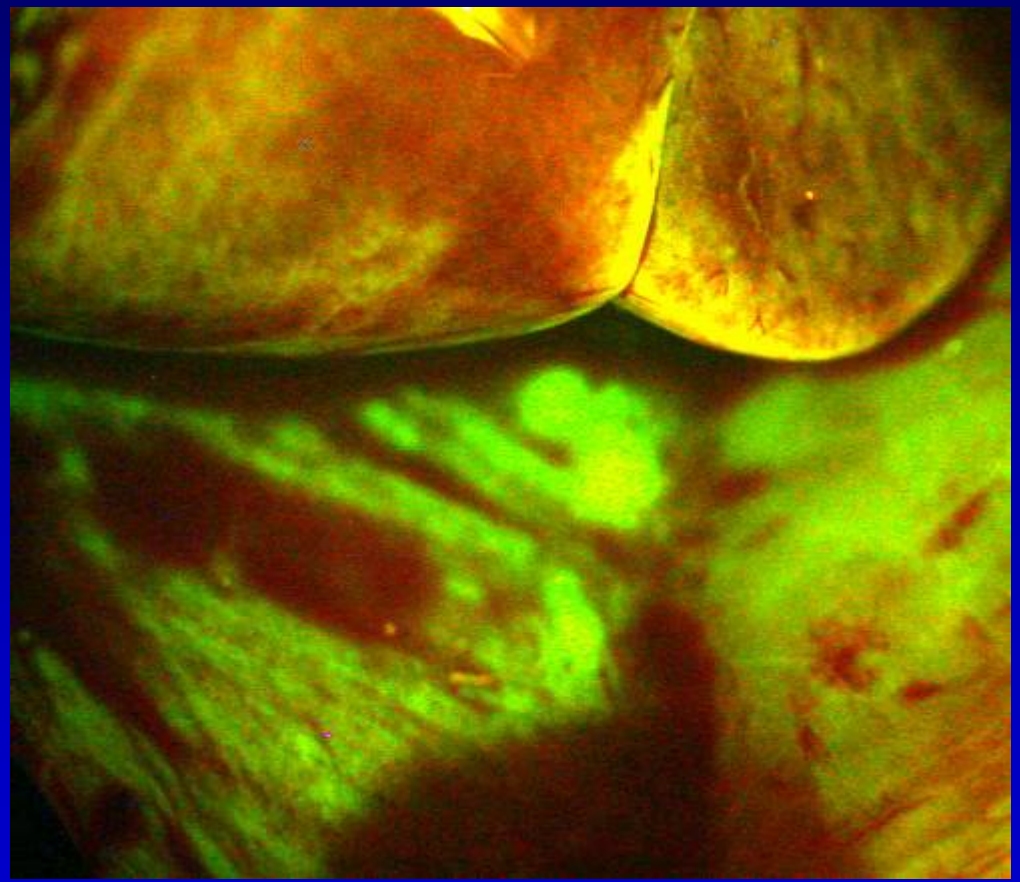
Reduced death rate?

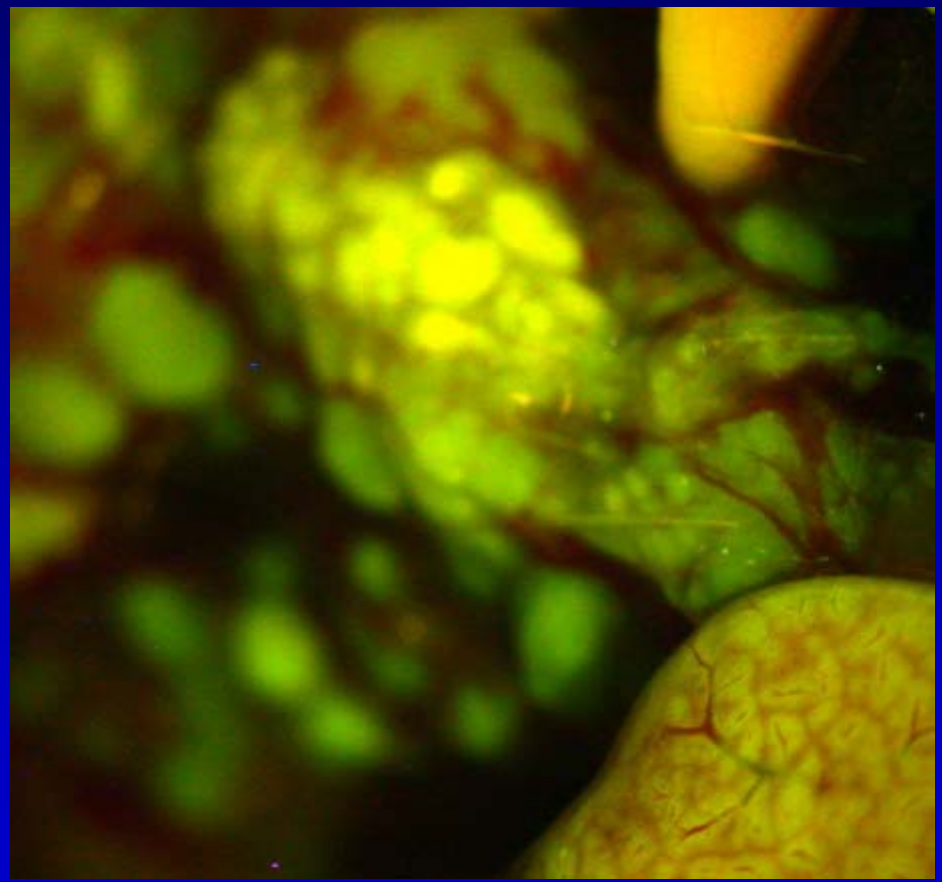
Tools: lentiviral BARD1



Tet ON BARD1
Tet ON BARD1-GFP
CMV BARD1 iresGFP
CMV BARD1-GFP
Tet ON BARD1 iresGFP

Tools: Nutu-GFP cells
Monitoring of metastatic spreading





Liver and peritoneum

Immune therapy and gene therapy