DNA DAMAGE-INDUCED RESPONSES

• Cell Cycle Checkpoints
• Transcriptional Regulation of Genes
• Telomere Length
• DNA Repair Mechanisms

• Apoptosis

Apoptosis #6644
DNA Damage-induced Cell Death

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Biochemical Mechanisms of Apoptosis

1. Death signals
   Receptor mediated
   Non-receptor mediated

   Genetic programming, nucleus initiates suicide response

2. Integration or control stage
   Signal transduction, Activation of transcription factors, Induction of apoptosis related genes, Release of Ca^{+2}, Depletion of ATP

3. The execution stage
   Cell dismantling, DNA degradation, Expression of phagocyte recognition molecules

4. Removal of dead cells by phagocytosis

Extrinsic and intrinsic apoptotic pathways
Role of tumor suppressor p53 in DNA damage-induced apoptosis

- p53 is required for apoptosis after some stimuli that introduce DNA damage.
- p53 is an important tumor suppressor whose loss is associated with rapid tumor development.
- It is a transcription factor and can regulate expression of Bcl-2, Bax and other Bcl-2 family members.

p53 integrates the cell cycle and apoptosis

- γ-irradiation leads to DNA damage.
- p53 activates cell cycle arrest and DNA repair.
- p53 can also lead to irreversible DNA damage and apoptosis.

(Optimization of damaged and potentially cancerous cells)
Induction of growth arrest by p53

Is p53 involved in apoptosis?

Yes. p53 is required for γ-irradiation-induced apoptosis in mouse thymocytes
Lowe et al., Nature 362: 847-849
p53-mediated transcriptional regulation of apoptosis-related genes

- FasL/Trail
- Fas/Killer/DR5
- p53
- IGF-I
- IGF-IR
- IGF-BP3
- SST-dependent
- SST-independent
- p53 causes up-regulation of Bax and Bak and induces apoptosis in lung cancer cells

A

% Sub-G1 cells

H1299 H358 H322

Control Atp53 Δ3312

B

% TUNEL-positive cells

H1299 H358 H322

Control Atp53 Δ3312

Pearson et al., Clin. Cancer Res. 6: 887-890, 2000
Role of Bax to apoptotic response to anticancer agents

Bax gene mutations in colorectal carcinogenesis

0 Accelerates tumorigenesis with reduced apoptosis in Bax-/- mice.
0 Colon cancers of the microsatellite mutator phenotype.
0 >50% somatic frameshift mutations in the Bax gene.
More proposed mechanisms for p53
Induced apoptosis

0 Science 288: 1053-1058, 2000 (activation of NOXA)
0 Journal of Biological Chemistry 275: 16202-16212, 2000
(Direct mitochondrial localization)
0 Molecular Cell 11: 577-590, 2003
(direct interaction of mitochondrial p53 with Bcl-xL)
0 Cell 102: 849-862, 2000 (Activation of p53AIP1)
0 Molecular Cell 7: 673-694, 2001 (Activation of PUMA)
0 PNAS 100: 1931-1936, 2003 (PUMA-mediated apoptotic response to p53)

NOXA, a BH3-only member of the Bcl-2 Family and candidate of p53-induced apoptosis

Oda et al., Science 288: 1053-1058, 2000
NOXA, a BH3-only member of the Bcl-2 Family and candidate of p53-induced apoptosis

Oda et al., Science 288: 1053-1058, 2000

Noxa mRNA is increased in a p53 dependent manner in MEFS (IR, Ad-p53) and in thymocytes (IR) and the protein follow in thymocytes.

Q2: Does it promote death and act as a BH3 only family member?

Oda et al., Science 288: 1053-1058, 2000

Induces Apoptosis in BH3 dependent Manner

NOXA, a BH3-only member of the Bcl-2 family and candidate of p53-induced apoptosis

Oda et al., Science 288: 1053-1058, 2000
Death signal-induced localization of p53 protein to mitochondria during p53-independent apoptosis or during p53-mediated cell cycle arrest

Marchenko et al., JBC 275: 16202-16212, 2000
Death signal-induced localization of p53 protein to mitochondria during p53-independent apoptosis or during p53-mediated cell cycle arrest

Marchenko et al., JBC 275: 16202-16212, 2000

p53 interacts with Bcl-xL and induces apoptosis in mitochondria

p53AIP1 (p53-regulated apoptosis-inducing protein 1) is a potential mediator of p53-dependent apoptosis

Screened a human genomic library to look for novel targets of p53 using the Yeast enhancer TRAP. Identified and sequenced a novel gene and named it p53AIP1.

Northern blot analysis: p53AIP1 mRNA induced with p53 activation.

p53AIP1 is regulated but later than p21WAF1.

Oda et al., Cell 102: 849-862, 2000
**p53AIP1 mediated p53-induced apoptosis in T98G (glioblastoma) cells**

Oda et al., Cell 102: 849-862, 2000

**p53AIP1, a potential mediator of p53-dependent apoptosis and its regulation by Ser46-phosphorylated p53**

Oda et al., Cell 102: 849-862, 2000
**PUMA, a novel p53-induced proapoptotic gene**

A

[Biochemical sequence of PUMA and other proteins with SAGE analysis results]

B

Use SAGE analysis to identify p53 Targets: One target is PUMA (p53 upregulated modulator of apoptosis).

Other studies demonstrate that PUMA is activated by p53, binds to Bcl-2 and induces apoptosis when overexpressed.


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**PUMA and apoptosis of colorectal cancer cells**

[A diagram showing PUMA expression in HCT116 colorectal cancer cells under normal and adenovirus p53 (Ad-p53) conditions]

Yu et al., PNAS 100: 1931-1936, 2003
Conclusions:
For colon cancer cell lines: p53 dependent killing occurs via PUMA activation of Bax. For other cell types, other pathways may be involved

Yu et al., PNAS 100: 1931-1936, 2003

Role of PUMA and SLUG in modulating apoptosis mediated by p53

Cell 18: 123(4):545-548
Model for cellular decision to enter growth arrest or apoptosis in response to activated p53

Stress (UV, IR, others)

Activated p53

Low level p53

Low level MST

Low level SST

p21

Bax

NOXA

p53AIP

PUMA

Growth arrest

Senescence

survival

Rb

p21

p300

WT1

Apoptosis

SST-independent

High level SST

Mdm2

p21

Bax

NOXA

p53AIP

PUMA

SST

Growth arrest

Deregulated E2F1

Bcl-2↑

Mitogenic signals

cytokines

NORMAL CELL

CANCER CELL

Low level p53

Low level MST

Low level SST

p21

Bax

NOXA

p53AIP

PUMA

Growth arrest

Deregulated E2F1

Bcl-2↑

Mitogenic signals

cytokines