In most tumors - p53 is a late mutation

Why?

1) p53 is a checkpoint protein - it plays no role in absence of stress
So, p53 mutation doesn’t give selective advantage in a normal cell

2) But oncogenic signaling promotes arrest or apoptosis
This now gives strong selective pressure for p53 loss

Remember-

PROLIFERATION
APOTOPSIS
ARREST (also called Senescence)

Role of p53

p53

Cell cycle arrest
Apoptosis

How does p53 decide which one happens?
It depends on the cell/setting!!!

Role of p53

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THE CHOICE BETWEEN ARREST AND APOPTOSIS CAN BE MODULATED BY THE TUMORIGENIC EVENT
Think about activated ras or p16 loss versus pRB loss!
1) Aflatoxin-induced liver cancer

- Aflatoxin is a potent mutagen produced by Aspergillus
- It is the most potent carcinogen known
- It gets metabolized by cytochrome P450 system to form an electrophilic epoxide that attacks G and converts it to T
- A major target is codon 249 in p53
- In China - Aflatoxin is a major food contaminate
  - There is a very high incidence of liver cancer
  - 91% of cases have a G to T transition at codon 249

In a few cases, p53 mutation is an early event

2) Lung cancer in smokers

- The carcinogenic chemical in tobacco (Benz(e)pyrene) is also gets metabolized by cytochrome P450 system to form an electrophilic epoxide that attacks G and converts it to T
- A major target is codon 249 in p53
- In smokers - almost always see mutation of codon 249
  - This is present in the earliest lesions
- In non-smokers - the incidence of codon 249 mutation is lower
  - it tends to be a later event

In a few cases, p53 mutation is an early event

3) Skin cancer

This is due to the other mechanism by which p53 is activated:

- DNA damage
- Oncogenic signaling

TUMOR SURVEILLANCE RESPONSE
DNA damage pathways

Stalled replication forks | Damaging agents
---|---
SSB | DSB
Detected: RPA | Mre11-rad50-NB1 (MRN) complex
Recruit: ATR | ATM
Mediate: Cell cycle arrest | Mark damage site
(Phosphorylation of H2AX to form gamma-H2AX)

Repair mechanisms

Stalled replication forks | Damaging agents
---|---
SSB | DSB
Detected: RPA | Mre11-rad50-NB1 (MRN) complex
Recruit: ATR | ATM
Recruit: FA complex Includes BRCA2
Includes BRCA1
Recruit: BASC complex
HR - Homologous recombination | Error prone repair
NHEJ - Non-homologous End-joining

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Key Commitment Points

Segregation of chromosomes to daughter cells

BOTH ARE CHANCES TO INTRODUCE ERRORS

CELL CYCLE ARREST PREVENTS PROGRESSION THROUGH THESE POINTS

Cell cycle arrest mechanisms

<table>
<thead>
<tr>
<th>Stalled replication forks</th>
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<td>Recruit:</td>
<td></td>
</tr>
<tr>
<td>ATR</td>
<td>ATM</td>
</tr>
</tbody>
</table>

Inhibition of cdk/cyclin complexes by:

1) Inhibition of cdc25  
2) Activation of p53

Regulation of p53

Normal cell

Mdm2  Oncogene

p53   Tumor suppressor
Regulation of p53

Normal cell

DNA damage

ATM + ATR

Mdm2

chk1 + chk2

Mdm2

p53

p53\textsuperscript{p}

Cell cycle arrest

Apoptosis

Phosphorylation of p53 prevents it from binding to Mdm2

Regulation of p53

Normal cell

DNA damage

ATM + ATR

Mdm2

chk1 + chk2

Mdm2

p53\textsuperscript{p}

Cell cycle arrest

Apoptosis

p53, ATM and chk2 are all tumor suppressors

Key Commitment Points

Segregation of chromosomes to daughter cells

BOTH ARE CHANCES TO INTRODUCE ERRORS

p53 loss prevents cell arrest/apoptosis in response to DNA damage

Segregation of chromosomes to daughter cells

Mitosis

G\textsubscript{2}

G\textsubscript{1}

S-phase

Replicate DNA

p53 loss prevents cell arrest/apoptosis in response to DNA damage
Evidence that p53 regulates apoptosis

2 mo. old littermates
Isolate thymocytes
Treat with gamma-IR

WT
Apoptosis
TUMOR CELL

p53-/

In a few cases, p53 mutation is an early event

3) Skin cancer
This is due to activation of p53 by DNA damage-

Keratinocytes
UV damage

WT
APOTOPSIS ARREST
DEAD CELL

p53-/

p53’s role in the DNA damage response influences the ability of tumors to respond to traditional treatments
1) RADIATION

Mechanism:

\[ H_2O \rightarrow OH^- \]

DNA-sugar backbone

s.s.breaks > d.s. breaks

How much damage?

Cell studies:

1 gray/cell - 1000 s.s.breaks
40 d.s. breaks

Therapeutic dose - 20 to 50 grays

Advantages:

• Localized dose (use MRI to locate tumor and 3 beams to limit damage to normal tissues)
• Preserves anatomy

Disadvantages:

• Don’t recover tumor to check pathology
• Localized - does not target metastases
2) Classic Chemotherapeutics

4 general mechanisms-

- Alkylating agents
- Topoisomerase inhibitors
- Anti-metabolites
- Microtubule inhibitors

- Alkylating agents

- Topoisomerase inhibitors
Anti-metabolites
Chemical analogues of cofactors for key metabolic enzymes
E.g. methotrexate - resembles folic acid

Microtubule inhibitors
Remember - chromosome segregation requires formation of mitotic spindle

Chemotherapy
Advantages -
SYSTEMIC - works on metastases
Disadvantages -
SYSTEMIC - also affects normal cells

Hair follicles
Gut lining
Haematopoietic stem cells -
Anemia (RBCs)
Infection problems (WBCs)
Clotting defect (platelets)
Radiation/chemotherapy response is dose-dependent
Tumor cells are more susceptible than normal

\[
\begin{align*}
\text{% survival} & \quad \text{Normal} \\
\text{Tumor} & \quad \text{Chemotherapeutic} \\
\text{or radiation dose} & \quad \text{dose}
\end{align*}
\]

This difference defines the therapeutic window

\[
\begin{align*}
\text{% survival} & \quad \text{Chemotherapeutic window} \\
\text{Tumor} & \quad \text{Normal} \\
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\end{align*}
\]

The chemotherapeutic window is small

Cause of specificity

Original hypothesis
- Chemotherapeutics target rapidly dividing cells
- Tumors cells divide most rapidly than normal
**Cause of specificity**

**ORIGINAL HYPOTHESIS**
- Chemotherapeutics target rapidly dividing cells
- Tumors cells divide most rapidly than normal

**BUT this can’t be the whole answer because**-
- Many FAST DIVIDING tumors are RESISTANT
- Many SLOW DIVIDING tumors are SENSITIVE
- Many normal cells divide at the same rate as tumor cells but they are typically more RESISTANT

**CURRENT HYPOTHESIS**
- Chemotherapeutics induce DNA DAMAGE and therefore APOPTOSIS
- Tumors cells have a higher predisposition to apoptosis than normal cells

**Evidence that chemotherapeutics induce apoptosis**

Isolate wild-type MEFs

Select cell lines

Treat with chemotherapeutics

Control retrovirus → ras

Activated ras + c-myc → apoptosis

LIVES → DIES
This is actually dose-dependent. Tumor cells are more sensitive than normal cells.

\[
\text{Chemotherapeutic dose} \quad \begin{array}{c}
\text{Normal} \\
\text{Tumor}
\end{array}
\]

SAME SPECIFICITY WITH RADIATION!!

This is similar to the differential response of normal and tumor tissue to chemotherapy or radiation.

\[
\text{Therapeutic dose} \quad \begin{array}{c}
\text{Therapeutic window} \\
\text{Normal} \\
\text{Tumor}
\end{array}
\]

**Mechanism of apoptosis**

\[\text{Chemo/radiation} \downarrow \text{DNA damage} \]

\[\text{ATM + ATR} \quad \text{chk1 + chk2} \]

\[\text{p53} \]

\[
\text{Cell cycle Arrest} \quad \begin{array}{c}
G_1/S \gg G_2/M
\end{array}
\]

Apoptosis
In most cell types-

LOW level DNA damage → Apoptosis

HIGH level DNA damage → Apoptosis

Why arrest?

Remember there are some exceptions-
E.g. In keratinocytes - apoptosis is the default consequence

WT p53 mutation is an essential (early) event for tumorigenesis

UV damage → Apoptosis

p53-/ TUMOR CELL

Arrest >> apoptosis

Apoptosis >> Arrest
Cell cycle Arrest
\( G_1/S \rightarrow G_2/M \)
Apoptosis

Chemo/radiation

DNA damage

ATM + ATR
chk1 + chk2

DNA Repair machinery

p53

Activate p53
G1/S or G2/M arrest
Repair DNA
p53 no longer activated
Cell cycle can continue

Why do tumor cells respond differently from normal cells?

Chemo/radiation

DNA damage

ATM + ATR
chk1 + chk2

p53

Cell cycle Arrest
\( G_1/S \rightarrow G_2/M \)
Apoptosis
In NORMAL cell types -

- LOW level DNA damage leads to Arrest >> apoptosis
- HIGH level DNA damage leads to Apoptosis >> Arrest

In TUMOR cells -
balance is shifted towards apoptosis

- LOW level DNA damage leads to Arrest >> apoptosis
- HIGH level DNA damage leads to Apoptosis >> Arrest
i.e. you need less damage to activate the apoptotic response

Reason #1

- Oncogene activation
- p53 is being activated through 2 pathways!!!

Chemo/radiation

- DNA damage
- ATM + ATR
- chk1 + chk2

Cell cycle Arrest

- G1/S >> G2/M

Apoptosis
Reason #2

- Oncogene activation
- DNA damage
- ATM + ATR
- chk1 + chk2
- The G1 arrest pathway is compromised
- p53
- Apoptosis

Cell cycle Arrest $G_1/S \gg G_2/M$

**Mechanism of $G_1/S$ arrest**

- p21 inhibits pRB kinases
- NO activation of E2F
- Arrest at $G_1/S$

WT cells:
- p21 inhibits pRB kinases
- NO activation of E2F
- Arrest at $G_1/S$

Tumor cells:
- pRB is functionally inactivated
- Ability of p21 to inhibit E2F is impaired

**In the absence of a functional $G1/S$ checkpoint the balance is shifted towards apoptosis**

- p53
- Cell cycle arrest
- Apoptosis

In the absence of a functional $G1/S$ checkpoint the balance is shifted towards apoptosis.
Evidence that pRB status influences cell cycle arrest in response to chemotherapy

Isolate MEFs

Treat with various doses of chemotherapeutic agents

48 hrs later - assay arrest by incubating with BrdU for 12 hrs

Evidence that pRB status influences cell cycle arrest in response to chemotherapy

Selectivity arises because tumor cells have an increased susceptibility to apoptosis instead of arrest
Selectivity arises because tumor cells have an increased susceptibility to apoptosis instead of arrest.

BUT - apoptosis requires p53, and 50% of tumors have mutant p53.

p53-status has a major effect on cellular response to chemotherapeutics.

WT
Control retrovirus
Select
WT
Activated ras + c-myc
Treat with drugs

Apoptosis
G1 arrest

WT
Transformed

WT
p53-/-

Transformed
Resistant to arrest
And apoptosis
p53-status has a major effect on cellular response to chemotherapeutics

% survival

Chemotherapeutic dose

Normal

Tumor mutant p53

Tumor WT p53

p53-status in animal models

MEFs

WT

Activated ras + c-myc

WT

WT

p53-/-

p53-/-

Inject 10^5 cells into flank

Let tumor develop

p53-status has a major effect on cellular response to chemotherapeutics

Does p53 status affect response in:

- Animal models?
- Human patients?
Treat mice with chemotherapeutic agent

Animal models - YES

Human patients -
Strong correlation between response to chemotherapeutic agents and p53 status

E.g. WILM'S TUMOR

<table>
<thead>
<tr>
<th>Response</th>
<th>p53 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>95%</td>
<td>++++</td>
</tr>
<tr>
<td>5%</td>
<td>+/-</td>
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</table>

8/10 - detect p53 mutations

Conclusions

Patients with wild-type p53 tend to have a GOOD prognosis

Patients with mutant p53 tend to have a POOR prognosis

Even in tumor with wild-type p53-
p53 status can have a profound affect in the success of treatment
Remember this experiment!

MEFs

- Activated ras + c-myc
- Inject 10^5 cells into flank
- Let tumor develop

Transformed WT

Transformed p53^/-

Tumor size

WT

p53^/-

Tumor size

+ drug

Time (days)

+ drug

Time (days)

Treat mice with chemotherapeutic agent

W/T

p53^/-

Tumor size

drug

Time (days)
Tumor size

WT

Time (days)

Drug treatment

Find that p53 is now mutant

Because the treatment causes DNA damage it helps promote p53 inactivation

The chemotherapeutic window is SMALL

BUT - you want to give the highest dose possible to have the best chance of eliminating the tumor with the first treatments

% survival

Chemotherapeutic dose

Chemotherapeutic window

Normal

Tumor

SHOULD WE DETERMINE p53 STATUS BEFORE TREATMENT?

CONSIDERATIONS-

• Is there a downside to treating p53-deficient tumors with chemotherapy?
  Yes - promote more damage!!

• Are there alternative treatments?
  This is a major goal of the cancer field!!