p53 Status In Human Cancers; Therapeutic Opportunities

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p53

Molecule of the year 2003 (Science)

16th International p53 workshop
Stockholm Waterfront Congress Centre, June 15-19, 2014
p53 mutation frequency in somatic cancers

Figure by: Soussi T, Wiman KG Cell Death Differ. 2015 Aug;22(8):1239-49.

Based on: The Cancer Genome Atlas Pan-Cancer analysis project
The discovery of p53 -1979

Linzer DI, Levine AJ (1979), Cell 17: 43–52

Lane DP, Crawford LV (1979) Nature 278: 261–263


Cloning

Immunological
Findings

Cloning

SV40

T antigen

X = 53 kDa

cancer cell

Immunological

SV40

T antigen

X = 53 kDa

p53

X = 53 kDa
What does p53 do?

• The battle was on....


p53 + other oncogene(s) drive tumourigenesis

p53 = oncogene
However...

• Loss of p53 leads to tumourigenesis

• Genomic rearrangements

p53 ≠ oncogene
p53 = tumour suppressor


p53 was lost in many human cancers, p53 has anti-proliferative capacity
Tumour suppressor


p53 +/+  p53+/- or p53-/-  lymphomas
How?
p53 function

Cell cycle arrest/
DNA repair

p53

Mut p53

RIP
Many more targets...
p53 regulation

DNA damage

UV stresses

ATM

ATR

ARF

Ras oncogenes

p53

MDM2

p53 degradation
Many more activation mechanisms

Mutant p53

- Apoptosis
- Cell cycle arrest
- Invasion/metastasis
- Proliferation
- Decreased apoptosis/Chemo-resistance

GOF (Gain-of-function)
Mutant p53 as an Oncogene

p53 -/+ or p53 -/-

↓

Lymphomas (Sarcomas)

p53R172H/+ ->

↓

Lymphomas Sarcomas Carcinomas

Metastases

Olive KP et al, cell 2004 Dec 17; 119(6):847-60
Lang GA et al, cell 2004 Dec 17;119(6):861-72
Mutant p53 Gain Of Function

1. Mutant p53
   - CoFactor
   - mp53 site?/centrosome/MAR

2a. Mutant p53
    - CoFactor
    - TF
    - TF binding site

2b. Mutant p53
    - stimulus
    - CoFactor
    - TF
    - TF binding site

3. Mutant p53
   - CoFactor
   - TF
   - aggregation?

4. Mutant p53
   - TF binding site
   - X

My lab

mutant p53

Cell Motility

Drug Resistance

Cell engulfment
Cell Engulfment

Time lapse

IHC

H&E

mt p53 staining
p53 based therapy

p53 status not routinely determined
Only limited usage of p53 based therapy in clinic
>150 trials in NCI trial database involving p53

However, several strategies/ molecules are in clinical trial
### p53 based therapy

<table>
<thead>
<tr>
<th>no p53</th>
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<tbody>
<tr>
<td>adenovirus</td>
<td>p63/ p73</td>
<td>CRM1 (nuclear export)</td>
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- CRM1: CRM1 (nuclear export)
- downstream signaling: CRISPR-Cas9
- vaccination: Vaccination
If p53 still there...

- Not activated – (activating/ stabilizing drugs)
- Too fast degraded – (stabilization)
- Not localised in nucleus – (inhibition nuclear export)
- No downstream signaling (more like p53 null)
- Unfolded (more like mutant p53)
Preventing degradation – MDM2 inhibition
Preventing degradation-indirect MDM2 inhibition

- Nucleolar disruption
- Ribosomal stress

CDK inhibitors -
Roscovitine, Flavopiridol

Ribonucleotide production inhibitors -
PALA, Pyrazofurin

RNA pol inhibition -
Actinomycin D
MDM2 redundancy and stability
Inhibiting nuclear export
Inhibiting inhibitors/ others

![Diagram showing histone deacetylase inhibitors and their interaction with SIRT1/2, acetylation (ac), and p53.]

- Histone Deacetylase Inhibitors: Cambinol, Sirtinol, Suramin, Tenovins
- SIRT 1/2
- ac
- p53
- p53 Interacting Molecules: CP-31398, P-28
p53

- Try stabilizing and activating
- In normal cells – senescence
- In tumour cells – apoptosis
- Non-specific, side-effects
p53 based therapy

- no p53
- p53
- mutant p53

- p63/p73
- CRM1 (nuclear export)
- proteasome degradation
- inhibitors

- adenovirus

- interaction
- conformation change
- downstream signaling

- vaccination
No p53

• p53 family members still present?
  – Activation of p73 and p63

• Adenoviral therapy
  – gene restoration
Activating p53 family members
Similar regulation (p73)
p63

HISTONE DEACETYLASE INHIBITORS

Camphinol
Sirtinol
Suramin
Tenovins

SIRT 1/2

ac

p63
Adenoviral therapy

Adenovirus

Advexin/ Gendicine
Onyx015

cancer cell
No p53

- p63/ p73
  - non-specific
- Viral
  - low efficiency
p53 based therapy

- **no p53**: p63/p73
  - adenovirus

- **p53**: p63/p73
  - CRM1 (nuclear export)
  - proteasome degradation

- **mutant p53**: interaction
  - proteasome degradation
  - mutant p53
  - conformation change
  - downstream signaling
  - vaccination

- **inhibitors**

- **vaccination**
Mutant p53

- Which class of mutation? Reactivating molecules
- Mutant p53 stabilized? Proteasomal degradation
- Reactivation of family members? Inhibition of p63 and/or p73
- Vaccination
- Inhibition of downstream GOF pathways
Re-activating compounds

Conformational mutants

DNA contact mutants


c1b3

p53

mutant p53

mutant p53

PERACTIVATING COMPOUNDS

C-terminal peptides,
9HE,
CP-31398,
CDB3,
WR2721,
PRIMA1-Met,
Mira-1,
Phikan083,
NSC319726,
STIMA-1,
SCH529074,
Maleimide analogs
PK7088
Mutant p53 stabilization

CM5 p53 staining

Lung adenocarcinoma

proteasome degradation
Mutant p53 dependence in tumours

Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment


tam p53R248Q/-
No tamoxifen
Tumour regression!!!!
Mutant p53 proteasomal degradation
Mutant p53 autophagy

Autophagy

↓ glucose
Nutrient deprivation
HDAC inhibitors?
The p63/p73 mutant p53 interaction

- Apoptosis
- Invasion
- Metastasis

PREVENTING INTERACTION RETRA
Vaccination

Anti-p53

Recombinant virus
Inhibiting downstream signaling pathways

- Mutant p53
- SREBP
- Mevalonate
- Cholesterol
- Statins
Inhibiting downstream signaling pathways (2)
My research...

Random motility

control

Mutant p53- 273H

Muller P, Cell. 2009 Dec 24;139(7):1327-41
RCP dependent

EGFR  integrin  MET  MDR

TAp63

Mutant p53

random motility invasion  scattering/invasion  chemo/drug resistance

RCP
RCP and chemoresistance

survival

Concentration doxorubicin

si ctr
si RCP

doxorubicin²
RCP dependent

Inhibitors

EGFR  integrin  MET  MDR

random motility invasion  scattering/invasion  chemo/drug resistance

Mutant p53  TAp63
Mutant p53

• More specific
• Good potential
• Is degrading mtp53 enough? (long term)
• Inhibiting downstream pathways enough?
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- *adenovirus*
- *CRM1 (nuclear export)*
- *proteasome degradation*
- *inhibitors*
- *downstream signaling*
- *vaccination*

- *interaction*
- *conformation change*
Difficulties

– Isoforms
– Modifications
– Different mutations
– p53 plays an important role in many processes - unpredictability in response
Future directions

• Synthetic lethality
  – Exploit tumour vulnerabilities

• Novel strategies to correct mutations
  – Trans-splicing repair
Synthetic lethality

p53 in breast cancer subtypes and new insights into response to chemotherapy

Philippe Bertheau, Jacqueline Lehmann-Chê, Mariana Varna, Anne Dumay, Brigitte Poirot, Raphaël Porcher, Elisabeth Turpin, Louis-François Plassa, Anne de Roquancourt, Edwige Bourstyn, Patricia de Cremoux, Anne Janin, Sylvie Glacchetti, Marc Esplé, Hugues de Thé

doi:10.1016/j.breast.2013.07.006
Trans-splicing repair

Hepatocellular cancer cells
Sci Rep. 2015 Mar 3;5:8705

Colorectal cancer cells
Oncotarget. 2015 Feb 10;6(4):2034-45
Conclusions

• Progress is being made
• Better understanding of different mutations, cell specificity, tumour specificity, tumour characteristics etc
Acknowledgements