FUNCTIONS and REGULATION OF THE p53 TUMOR SUPPRESSOR PROTEIN

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The programme of this talk:

- regulation of p53 activity, especially by oncogenic activation
- induction of apoptosis as tumor suppressor activity
- regulation of metabolism as tumor suppressor activity

- functional inactivation of wt-p53 in tumors by MDM2/MDMX
  - potential to reactivate wt-p53 as therapeutic intervention
- functions of mutant p53
  - potential to reactivate mt-p53 as therapeutic intervention
Most patients of the cancer-prone Li-Fraumeni Syndrome (LFS) carry a heterozygous p53 gene mutation
How can the p53 protein perform its tumor suppressor function(s)?

**p53 = transcription factor:**

Mainly performs its function by regulation of gene transcription

> both activation and repression of transcription

> protein coding genes (mRNAs)
> non protein coding genes, like miRNAs, eRNAs and lncRNAs

In normal proliferating and differentiated cells,

> p53 protein levels are low
> p53 has low activity

Many forms of stress can stimulate p53 activity:

> increase in p53 protein expression levels
> post-translational modifications enhancing p53 activity
  (phosphorylation/acetylation/methylation/ubiquitination/sumoylation)
> increase in p53 protein level upon stress is result of prolonged half-life

- p53 protein stability is primarily regulated by the MDM2 protein, in a ubiquitin- and proteasome-dependent manner
Regulation of p53 stability

> increase in p53 protein level upon stress is result of prolonged half-life

- p53 protein stability is primarily regulated by the MDM2 protein, in a ubiquitin- and proteasome-dependent manner

In normal unstressed cells,

> MDM2 binds p53,
> ubiquitinates p53

> targets p53 for degradation by the proteasome, resulting in low basal p53 protein levels

Identification of MDM2 family member: MDMX
MDM2 and MDMX: essential p53 inhibitory proteins

Montes de Oca Luna et al., Nature 1995
Jones et al., Nature 1995

Parant et al., Nat. Genet. 2001
Finch et al., Cancer Res. 2002
MDM2 and MDMX in the regulation of p53

Marine JC, Dyer MA, Jochemsen AG.

Wild-type p53 is tumor suppressor protein:

how is p53 activated upon oncogenic stress?
Oncogene activation/loss of tumor suppressor function (pRB) leads to increased expression of transcription factors DMP1 and/or E2F1.

DMP1 and E2F1 enhance transcription of the p14ARF gene resulting in an increase in p14ARF protein levels.

p14ARF interacts with MDM2, thereby inhibiting its ubiquitin-ligase activity leading to stabilization and activation of p53.

Adapted from: Soussi & Béroud, Nature Reviews Cancer 1, 233-239, 2001
Wild-type p53 is tumor suppressor protein:

Via which biological processes is p53 performing its tumor suppressor functions?
The classical view of p53 activation and response

Role for $p14^{ARF}$-dependent, $p53$-induced apoptosis in tumor protection after oncogene activation
Transgenic mice with transcription of the c-Myc oncogene under transcriptional control of the Enhancer of the IgM Heavy Chain gene (Eμ):

> B-cell specific expression
Role of p53-induced apoptosis in c-myc induced lymphomagenesis

Eµ–myc/wt

Long latency caused by high level of apoptosis in the hyperproliferating B-cells

Schmitt et al, Genes Dev 13, 1999
Role of p53-induced apoptosis in c-myc induced lymphomagenesis

Schmitt et al, Genes Dev 13, 1999

Eµ–myc/WT
Eµ–myc/p53+/-

Strong reduction in the level of apoptosis in the hyperproliferating B-cells
Role of p53-induced apoptosis in c-myc induced lymphomagenesis

Strong reduction in the level of apoptosis in the hyperproliferating B-cells

Eµ–myc/wt
Eµ–myc/p53+/-
Eµ–myc/p14ARF+/-

Schmitt et al, Genes Dev 13, 1999
Role of p53-induced apoptosis in c-myc induced lymphomagenesis

Schmitt et al, Genes Dev 13, 1999

Strong reduction in the level of apoptosis in the hyperproliferating B-cells
Conclusion:

Induction of apoptosis upon stimulation of inappropriate cell cycle progression (e.g. oncogene activation) is an important tumor suppressor function of p53.
The p53 protein can become activated by multiple forms of cellular stress and affects multiple cellular processes all aimed to protect the cells in the body from oncogenic transformation.

Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence

Cell culture studies: acetylation of 3 lysines in p53 is necessary for efficient induction of apoptosis and cell cycle arrest.

Generation of a mouse in which these 3 lysines are replaced by arginines.

Cells from p53/3KR mice are:
- resistant to p53-induced apoptosis (thymocytes)
- resistant to p53-induced cell cycle arrest (MEFs)

Cell culture studies: acetylation of 3 lysines in p53 is necessary for efficient induction of apoptosis and cell cycle arrest.

Generation of a mouse in which these 3 lysines are replaced by arginines.

but...

p53/3KR mice are NOT tumor prone.
The role of p53 in glucose metabolism
Eric C Cheung and Karen H Vousden

Current Opinion in Cell Biology 2010, 22:186-191

Diagram:
- Glucose
  - HK
  - G6PDH
    - Activate PPP
    - 2 NADPH
    - Ribose 5-phosphate
  - Glucose 6-phosphate
  - Fructose 6-phosphate
    - PFK1
- Fructose 1,6-bisphosphate
- 3-phosphoglycerate
- 2-phosphoglycerate
  - PGM
  - pyruvate
- TIGAR
- p53
  - Glut3
  - NF-kB
  - IKK
  - Glut1/4
  - AIF
  - SCO2
  - CI, CII, CIII, CIV

Annotations:
- Reduce glycolysis
- Maintain oxidative phosphorylation
- Reduce glucose uptake
The p53/3KR mutant can still regulate glucose metabolism

The p53/3KR mutant can still regulate glucose metabolism and suppress ROS levels like wt-p53.

Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence

Wild-type p53 as therapeutic cancer target

40-50% of human tumors still express a wild-type p53; attenuated tumor suppressor activity

Q: How is wild-type p53 inactivated in tumors?
Q: Can this wild-type p53 get re-activated?

MDM2 and MDMX are inhibitors of p53 activity:
  > an oncogenic function in tumors with wild-type p53?
**MDM2 as driver in human tumors**
Approximately 5% of all human tumors show overexpression of MDM2 (particularly sarcomas; up to 30%) 

> in general correlating with wild-type p53 status

**MDM2 as drug target**
High-throughput screen for inhibitors of p53/MDM2 interaction:
Nutlin-3: binds MDM2 within its p53-binding pocket
→ disruption of the p53/MDM2 interaction: → p53 activation!?
Nutlin-3 inhibits the growth of tumor cells with amplified MDM2 and wild-type p53, \textit{in vitro} and \textit{in vivo}


SJSA-1 = osteosarcoma cell line with amplified Mdm2
Clinical Trial with the ‘Nutlin’ RG7112 for WDLPS

WDLPS: Well Differentiated Liposarcoma

- Very frequent Mdm2 amplification
- Very sensitive to Nutlin-3 (and derivative RG7112) in cell culture

Ray-Coquard et al. study, using the Nutlin RG7112 on a schedule of daily dosing for 10 out of every 28 days, over 3 cycles, in 20 pre-operative MDM2-amplified primary WDLPS patients

The results showed that 14/17 patients attained stable disease, one patient achieved a partial response.

The study was correlated with grade 3 or 4 haematological toxicities as Adverse Effects (AEs).


The results showed that 14/17 patients attained stable disease, one patient achieved a partial response.

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Since a similar number of serious haematological AEs have been reported in other solid tumour RG7112 Phase I trials, haematological toxicity could be a serious limiting factor in the future clinical development of RG7112, as well as potentially for other clinical Nutlin compounds.

Alternative treatments to activate p53?

MDMX in tumor development

MDMX protein is found highly expressed in increasing number of tumor types, including retinoblastoma, breast carcinoma, several leukemias, sarcomas.

It has been shown that maintaining this high MDMX expression is needed for the proliferation and survival of retinoblastoma and breast tumor cells.

Cutaneous Melanoma

Driver mutation(s):

- BRAF (40-50%)
- N-Ras (15-25%)
- KIT (2-8%)

Low percentage of p53 mutations

Over 65% show very high levels of MDMX
Depletion of MDMX inhibits growth of metastatic melanoma cell lines; partly p53-independent

C = shCtrl
M = shMdmx
P = shp53
D = shMdmx/shp53

BrdU incorporation: measurement of S-phase cells

The *Mdmx* gene is expressed into two main splicing variants, the *Mdmx-FL* and *Mdmx-S* mRNAs.

The ratio between *Mdmx-FL* and *Mdmx-S* is highly variable between cell lines.

The small protein encoded by the *Mdmx-S* mRNA is very unstable (unfolded protein response?); thus essentially loss of MDMX protein expression.

The ratio between *Mdmx-FL/Mdmx-S* mRNA rather well predicts the level of functional MDMX protein expression.

Cutaneous Melanoma

Good correlation Mdmx-FL/Mdmx-S ratio and MDMX protein levels

Inducing the skipping of Mdmx exon 6 reduces MDMX protein levels, activates p53 and inhibits growth of metastatic melanoma cells in culture and in a PDX model

Inducing the skipping of $Mdmx$ exon 6 reduces MDMX protein levels, activates p53 and inhibits growth of metastatic melanoma cells in culture and in a PDX model, synergizes with mtBRAF-inhibition and prevents acquired BRAF-i resistance.

Conclusions on wild-type p53 activation

- MDM2 is overexpressed in proportion of human tumors, especially sarcomas.
- Targeting MDM2 to activate p53 is effective but yields strong Adverse Effects.

- Mdmx gene is frequently overexpressed/amplified in various tumors, including retinoblastoma, uveal melanoma, cutaneous melanoma and breast tumors and MDMX high protein expression is needed for their tumor cell survival.

- MDMX-overexpressing tumor cells are sensitive for decreasing the MDMX protein levels by modulation of Mdmx mRNA splicing via ASOs or small molecules targeting kinases involved in splicing.

Wild-type p53 is a valuable drug target in tumor cells overexpressing MDM2 and/or MDMX, particularly in combination therapies.
tumor analyses
- most human tumors expressing a mutant p53 gene retain the expression of this mutant p53 allele
  ➢ Suggests a selective advantage for mt p53

  ➢ Indeed, depleting mutant p53 from tumor cells strongly inhibits growth/survival

mouse models
p53 mt/- mice show a more diverse tumor spectrum, very frequent metastases and often multiple metastases
  › in contrast to p53 -/- mice
Conclusion

*MUTANT p53*: not only loss of wild-type function, but increases the invasiveness and metastatic potential of the tumors

**How to target mutant p53?**
# Mechanisms of oncogenic function of p53

## Conclusion

Mutant p53: not only loss of wild-type function, but increases the invasiveness and metastatic potential of the tumors.

## Table

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>Examples</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Mutant p53 interacts with DNA directly using mutant p53 binding elements or other regions on the DNA, including MARs, to regulate transcription. Transcriptional cofactors and other proteins can be involved.</td>
<td>PML, EGR1, TOP1, p300</td>
</tr>
<tr>
<td>2a</td>
<td>Mutant p53 enhances transcription by forming a complex with TFs that can include transcriptional cofactors and other proteins.</td>
<td>EGR1, TopBP1, PIN1, VDR, ETS1, NF-xB, p63, p73, SP1, SREBP, NF-Y, ETS2, E2F1, p300, HDAC, CBP</td>
</tr>
<tr>
<td>3</td>
<td>Mutant p53 decreases transcription by binding TFs and/or transcriptional cofactors and other proteins, sometimes preventing their binding to DNA. This activity can also involve aggregation of mutant p53 with other proteins.</td>
<td>TopBP1, ANKRD11, VDR, SMAD2, p63, p73, SP1, p300</td>
</tr>
<tr>
<td>4</td>
<td>Mutant p53 interacts with other proteins, not directly involved in transcriptional regulation, and enhances or blocks their function.</td>
<td>NRD1, EFEMP2, TOP1, BTG2, MRE11</td>
</tr>
</tbody>
</table>
Various approaches to target mutant p53

Cancer Cell. 2014; 25:304-17. Muller PA & Vousden KH.
Mutant p53 in cancer: new functions and therapeutic opportunities.
Reactivation of mutant p53

PRIMA interacts covalently with the core domain of p53
> changing conformation from mutant to wild-type

PRIMA reduces cell viability and tumor growth in mutant-p53 dependent fashion

Various approaches to target mutant p53

- Proteasome degradation
  - Mutant p53
  - HDAC inhibitors
    - Gambogic acid
    - Disulfiram
  - Conversion to wild-type p53
- Autophagy-mediated degradation
  - Mutant p53
- Glucose deprivation
- Nutrient deprivation
- Spautin-1
- HDAC inhibitors?

- Integration inhibitors?
- Statins?

- Interaction to other proteins
  - Mutant p53
  - Inhibition of downstream signaling pathways
  - Mutant p53

References:
In search for a compound destabilizing mutant p53

> p53-null cell line with mutant p53-luc fusion construct

Top-ten compounds further evaluated; only three consistently affected endogenous mutant p53: lovastatin, atorvastatine and mevastatin

Parrales A. et al.
Mevalonate pathway: the mt-p53 connection

Mevalonate pathway

Acetyl CoA

HMG-CoA Reductase

mevalonic acid

Mevalonate kinase

mevalonate-5-phosphate

Phosphomevalonate kinase

mevalonate-5-pyrophosphate

Farnesyl pyrophosphate

Protein prenylation

Cholesterol/steroid

Farnesylation: Ras!

Geranylgeranylation: Rho, Rac

CHIP = C-terminus of Hsc70 Interacting Protein
> ubiquitin ligase for 'misfolded' proteins..
Mevalonate pathway: the mt-p53 connection

- Statins
- Mevalonate pathway
- Mutant p53
- DNAJA1
- Activation YAP1/TAZ
- Proliferation and self-renewal

Freed-Pastor and Prives.
Nat Cell Biol 2016 18: 1122-1124
Conclusions on mutant p53 as cancer target

Mutant p53 increases tumor development, invasion and metastasis; by multiple mechanisms, e.g. inactivating the function of the p53 family members p63 and p73 and stimulating activity of oncogenic transcription factors

Re-activation or inhibition of mutant p53 is a valuable approach for cancer therapy particularly in combination therapies
Pharmacological re-activation of either wild-type or mutant p53 appears to be a promising approach for treatment of cancer patients, particularly in combination therapies.