FUNCTIONS and REGULATION OF THE p53 TUMOR SUPPRESSOR PROTEIN
The programme of this talk:

- introduction to p53 and its main regulators MDM2 and MDMX
- 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
Function(s) of p53?

\[ p53: \text{tumor suppressor gene} \]

- ~50% of human tumors contain a genetically altered p53 gene
- Most patients of the cancer-prone Li-Fraumeni Syndrome (LFS) carry a heterozygous p53 gene mutation
- p53 +/- and p53-/- mice develop tumors
How performs the p53 protein 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 (miRNAs, eRNAs and lncRNAs)

In normal proliferating and differentiated cells,

- p53 protein levels are very low
- p53 essentially has no basal activity

Many forms of stress can stimulate p53 activity:

- increase in p53 protein expression levels
- post-translational modifications modulating 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-mediated activation of p53 via p14<sub>ARF</sub>

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.

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

Schmitt et al, Genes Dev 13, 1999
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

- $E\mu-$myc/ wt
- $E\mu-$myc/p53$^{+/-}$

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

Role of p53-induced apoptosis in c-myc induced lymphomagenesis

Eµ–myc/mt
Eµ–myc/p53+/−
Eµ–myc/p14ARF+/−

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

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

Schmitt et al, Genes Dev 13, 1999
Conclusion:

Induction of apoptosis upon stimulation of inappropriate cell cycle progression (e.g. oncogene activation) is an important tumor suppressor function of p53.
A modern view of p53 activation and response

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

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 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

MDM2 and MDMX are inhibitors of p53 activity:

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, *in vitro* and *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. 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

Clinical Trial with the 'Nutlin' RG7112 for WDLPS

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 hematological toxicities as Adverse Effects (AEs).

Biswa S, Killick E, Jochemsen AG, Lunec J.
Clinical Trial with the ‘Nutlin’ RG7112 for WDLPS

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Since a similar number of serious hematological AEs have been reported in other solid tumor RG7112 Phase I trials, hematological toxicity could be a serious limiting factor in the future clinical development of RG7112, as well as for other clinical compounds focusing on disruption of the Mdm2/p53 interaction.

Alternative treatments to activate p53?

Biswas S, Killick E, Jochemsen AG, Lunec J.
MDMX in tumor development

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

It has been shown that maintaining this high MDMX expression is needed for the proliferation and survival of retinoblastoma, breast cancer and (Ewing) sarcoma 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
Over 65% of cutaneous melanoma show very high levels of MDMX

Depletion of MDMX with shRNAs inhibits growth of metastatic melanoma cell lines; partly p53-independent

How to target MDMX in the clinic?

Modulation of splicing with Anti-Sense Oligonucleotides

Targeting MDMX in cutaneous melanoma

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.

Targeting MDMX in cutaneous melanoma

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 BRAF-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 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
MUTANT p53: acts as an oncogene (?)

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
### Mechanisms of oncogenic function of p53

<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>
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<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-kB, 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>
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?**
Various approaches to target 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
  - HDAC inhibitors
  - Gambogic acid
  - Disulfiram
  - Mutant p53

- Conversion to wild-type p53
  - PRIMA-1
  - NSC319726
  - STIMA-1
  - SCH529074
  - CP-31398
  - Maleimide analogs
  - PK7088
  - PhiKan083
  - Zinc

- Mutant p53
  - ↓ glucose nutrient deprivation
  - Spautin-1
  - HDAC inhibitors?

- Mutant p53
  - Autophagy-mediated degradation

- Mutant p53
  - Interaction to other proteins

- Mutant p53
  - Inhibition of downstream signaling pathways
  - Integrin inhibitors?
  - Statins?

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

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/sterol

Farnesylation: Ras, Rho-B
Geranylgeranylation: Rho-A, Rho-C, Rac

CHIP = C-terminus of Hsc70 Interacting Protein > ubiquitin ligase for 'misfolded' proteins.

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

- Mutant p53
- DNAJA1
- CHIP
- Mutant p53

Statin pathway

Geranylgeranylation: Rho-A, Rho-C, Rac

Activation YAP1/TAZ

Proliferation and self-renewal


Re-activation or inhibition of mutant p53 is a valuable approach for cancer therapy particularly in combination therapies.