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Improving colon cancer therapy with a new promising small-molecule activator of the p53-pathway through disruption of p53-MDM2/MDMX interactions

Liliana Raimundo[1], Margarida Espadinha[2], Joana Soares[1], Joana B Loureiro[1], Juliana Calheiros[1], Nair Nazareth[1], Joana Almeida[1], Marco G Alves[3], Maria MM Santos[2] and Lucília Saraiva[1]

[1]LAQV/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal, [2]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, and [3]Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar & UMIB, Unity for Multidisciplinary Research in Biomedicine, University of Porto, Porto, Portugal

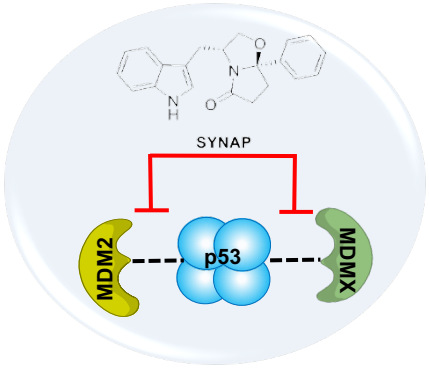
*lucilia.saraiva@ff.up.pt
*liliana-raimundo@live.com

Improving colon cancer therapy with a new promising small-molecule activator of the p53-pathway through disruption of p53-MDM2/MDMX interactions

p53-dependente antitumour activity in human tumor cells

- *Cell cycle arrest*
- *Apoptosis*
- *Migration inhibition*
- *p53 Transcriptional activity*
- *Non-genotoxic*

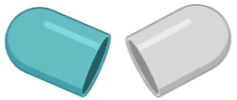
Mechanism of action



Disruption of the p53 interaction with MDM2/MDMX

p53 thermal stabilization

Synergy with conventional chemotherapeutic agents



SYNAP *Conventional drugs*

↑↑↑ *Antitumour effect*

No acquired or cross-resistance

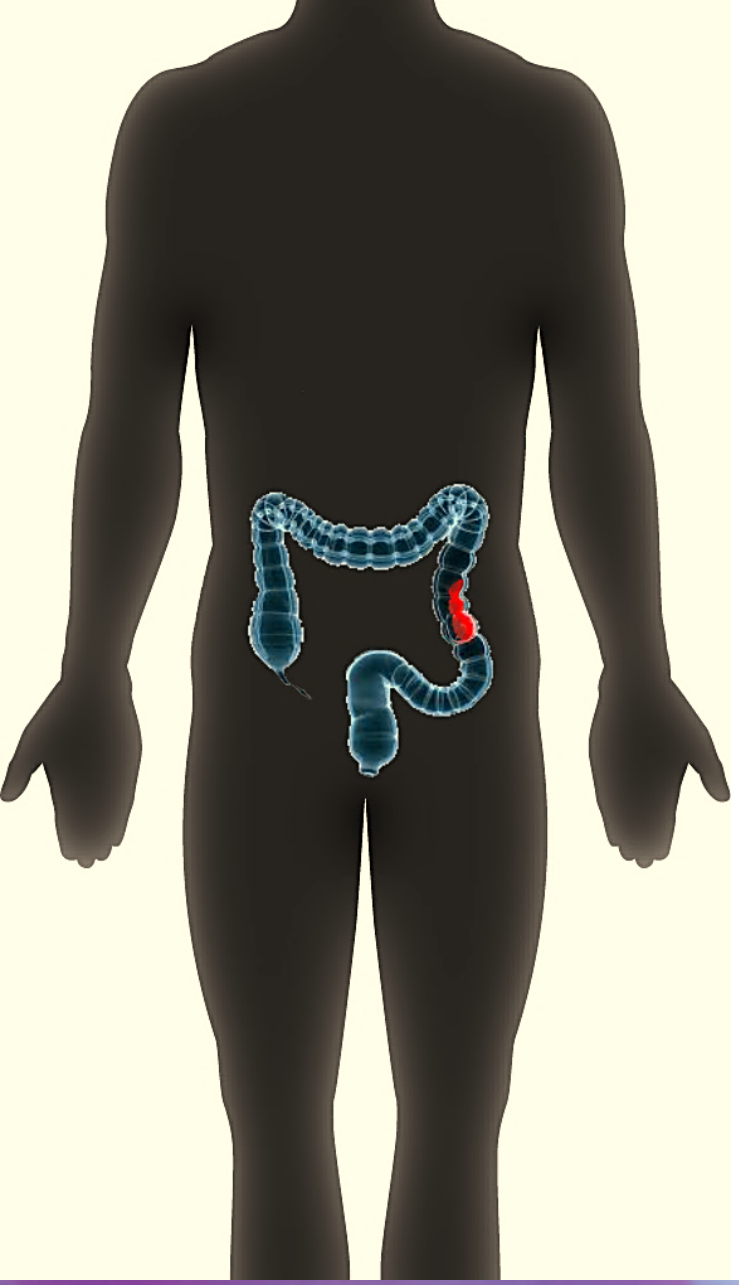
Re-sensibilization of doxorubicin-resistant cancer cells

Abstract

Impairment of the tumour suppressor p53 pathway is a major event in human cancers, making p53 activation one of the most attractive therapeutic strategies. This work describes the synthesis and biological evaluation of the (*R*)-tryptophanol-derived bicyclic lactam SYNAP as a selective p53 activator with potent anticancer activity against colon cancer. SYNAP anticancer activity and mechanism of action was studied in human colon adenocarcinoma HCT116 cells with wild-type p53 (HCT116p53$^{+/+}$) and the corresponding p53-null isogenic derivative cells (HCT116p53$^{-/-}$), presenting a potent anti-proliferative effect dependent on p53 status. In HCT116p53$^{+/+}$ cells, SYNAP p53-dependent growth inhibition was associated with cell cycle arrest, apoptosis, anti-migratory activity and upregulation of several p53 transcriptional targets. Data from a yeast-based assay and a co-immunoprecipitation assay in human cancer cells, indicated that SYNAP targeted p53 by inhibiting its interaction with murine double minute (MDM)2 and MDMX. Moreover, SYNAP sensitized colon cancer cells to the cytotoxic effect of known chemotherapeutic agents. In addition, SYNAP did not induce acquired or cross-resistance and re-sensitized doxorubicin-resistant colon cancer cells to the therapy. Importantly, SYNAP was non-genotoxic and presented low cytotoxic effects against normal cells.

Collectively, this work reports a new selective dual inhibitor of p53-MDM2/MDMX interactions with promising application in colon cancer therapy, both as monotherapy and in combination with known chemotherapeutic agents. Additionally, SYNAP represents a starting point for improved p53 activators, particularly inhibitors of p53-MDM2/MDMX interactions.

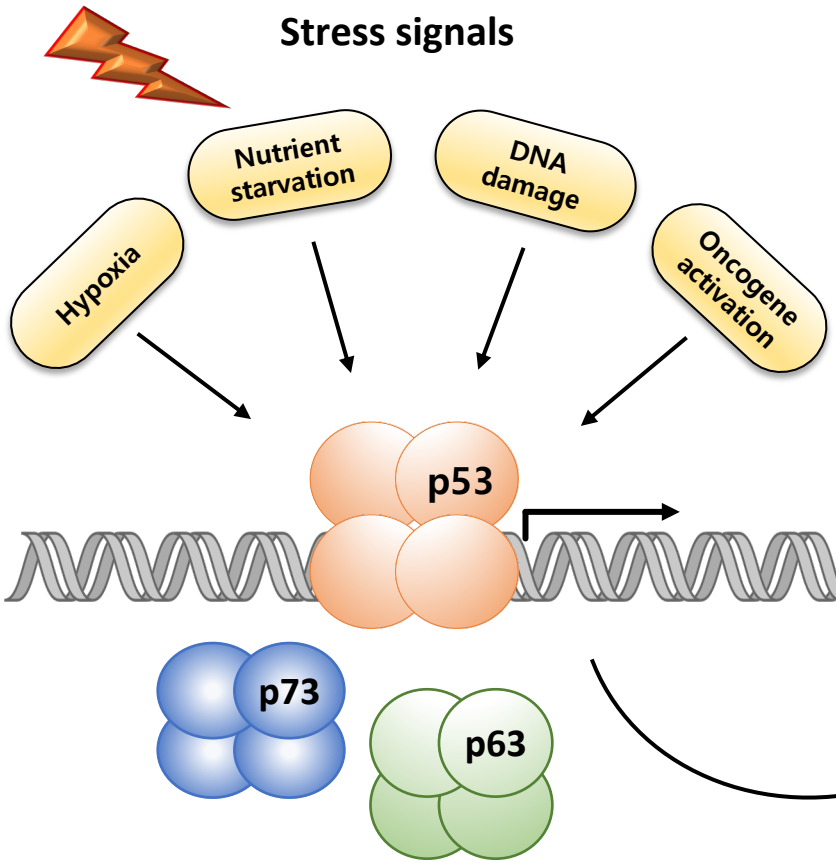
Keywords: p53 activation; inhibition of p53-MDM2/MDMX interactions; cancer treatment



Improving colon cancer therapy with a new promising small-molecule activator of the p53-pathway through disruption of p53-MDM2/MDMX interactions

- Colorectal cancer is a leading cause of cancer incidence/death worldwide
- 56% of colorectal cancer patients die from the disease
- Lack of effective treatments

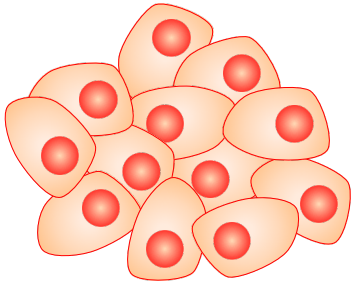
The p53 family of tumor suppressor proteins



p53 family proteins, p53, p63 and p73 are sequence specific **transcription factors**

- DNA Repair
- Cell Cycle Arrest
- Senescence
- Metabolic Reprogramming
- Apoptosis

Crucial role



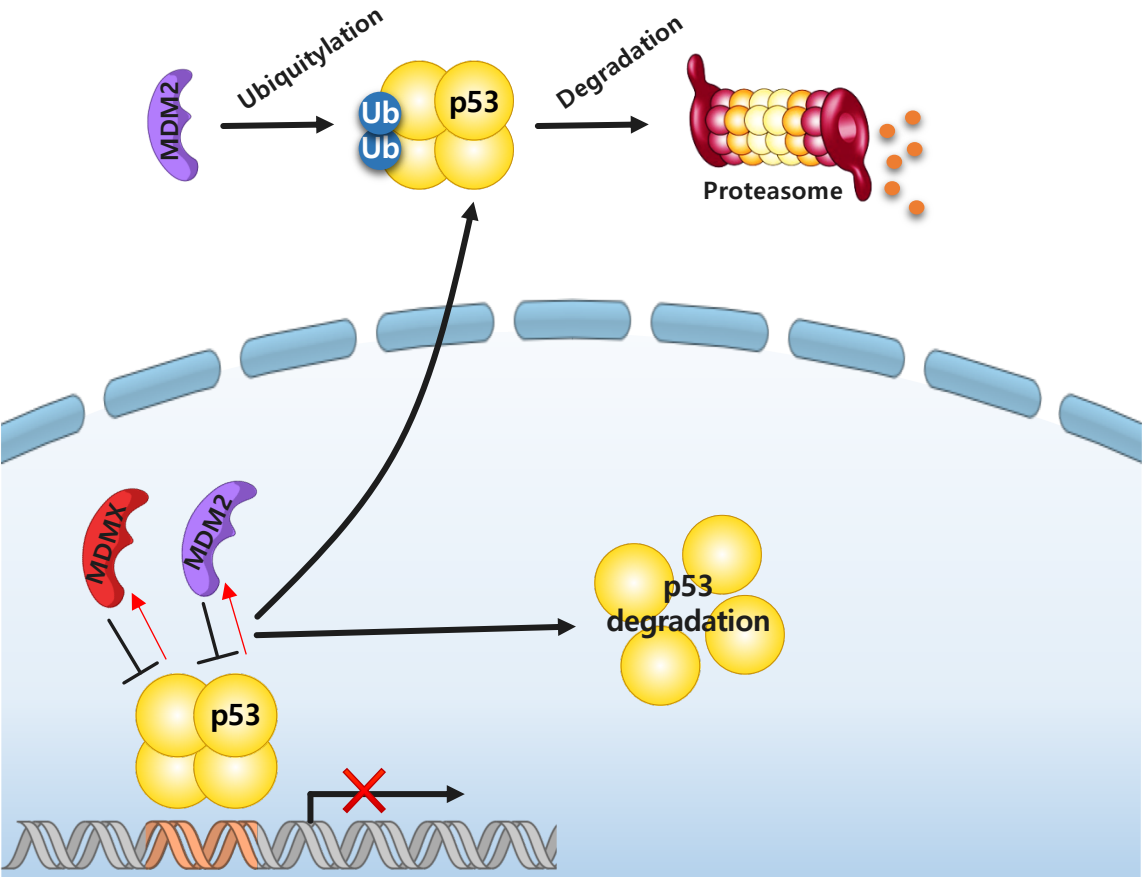
Tumorigenesis

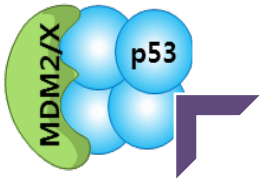
Targeting p53 family proteins: a valuable strategy in cancer therapy

Wei and Zaika (2012). J Nucl Acids 2012, 687359.; Kruiswijk F et al., Nat Rev Mol Cell Biol, 2015, 16:393-405.

Targeting p53 in cancer

Impairment of p53 function can be found in the majority of human cancers



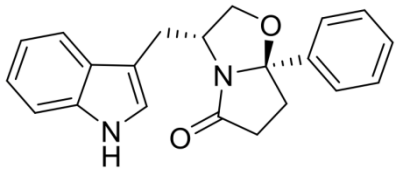


Inhibition of the p53-MDM2/MDMX interaction

DIMP53-1
OXAZ-1
Pyrrolopyrimidine 3a
Cis-imidazole derivative H109
Indolyl hydantoin (RO-5963)
Imidazoyl-indole (WK298)

Lenos and Jochemsen, J Biochem Biotechnology, 2011, 876173 Popowicz, *et al.,* Ang Chemie Inter, 2011, 50:2680-88.; Li, *et al.,* Cell cycle, 2010, 9:1411-20.; Li and Lozano, Clin Cancer Res, 2013, 19:34-41.; Hoe, *et al.,* Nat Rev, 2014, 13: 217-236

SYNAP has p53-dependent growth inhibitory effect in human cancer cells

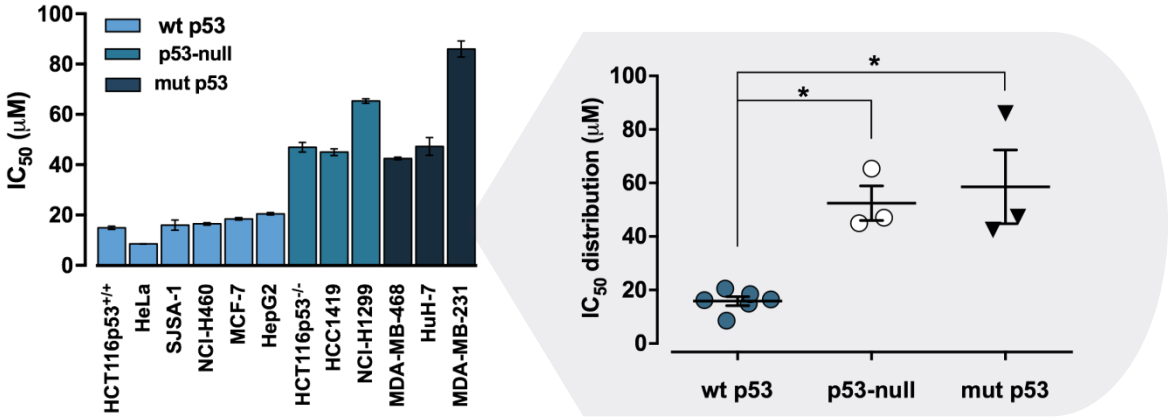


SYNAP

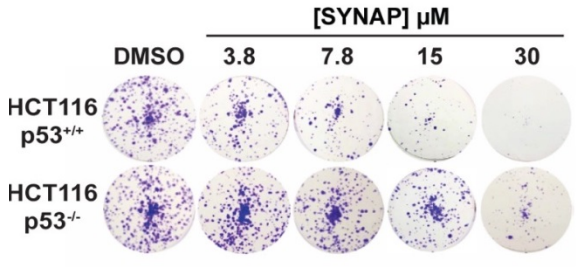
Doctor Maria MM
Santos Research Group

iMed.UL
Research Institute for Medicines and Pharmaceutical Sciences

Sulforhodamine B *assay*

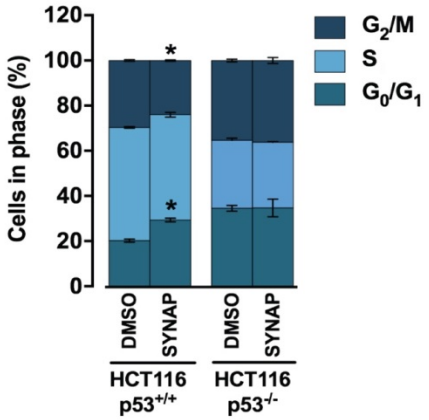


Colony formation assay



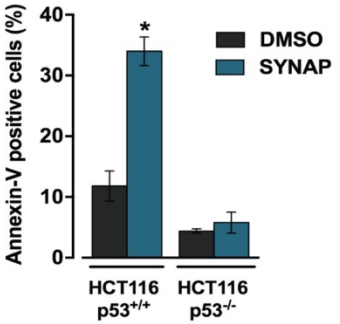
SYNAP has p53-dependent growth inhibitory effect in human colon cancer cells through induction of apoptosis and cell cycle arrest

Cell cycle analysis (PI)

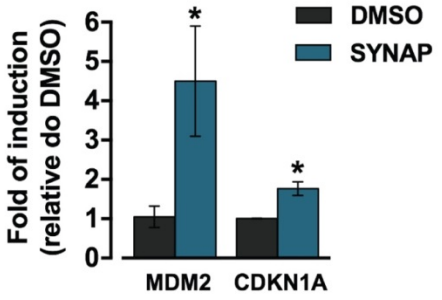


SYNAP was tested at 15 µM on HCT116 cells for 48h; Data are mean±SEM (n=5); *p<0.05

Apoptosis analysis (Annexin V/PI)

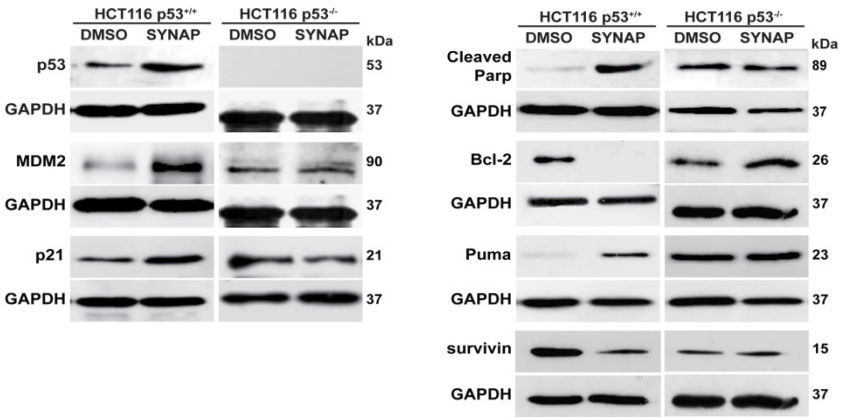


RT-qPCR analysis



SYNAP was tested at 15 µM on HCT116 cells for 48h; Data are mean±SEM (n=5); *p<0.05

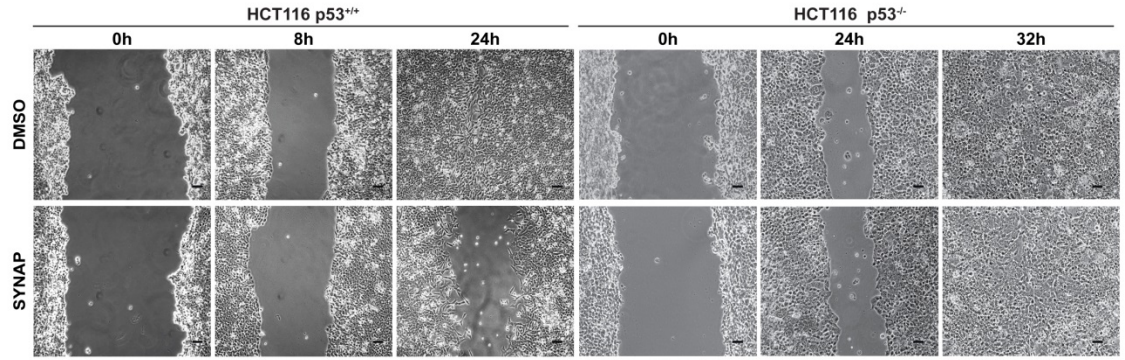
Western blot

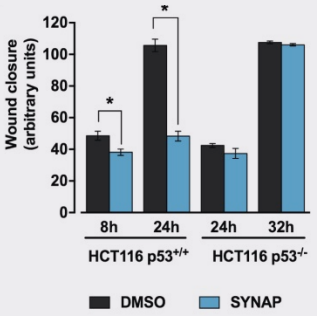


SYNAP was tested at 15 µM on HCT116 cells for 48h or 24h

SYNAP has p53-dependent anti-migratory activity in human colon cancer cells

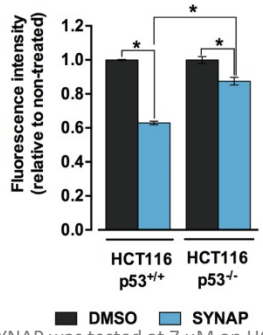
Wound healing assay





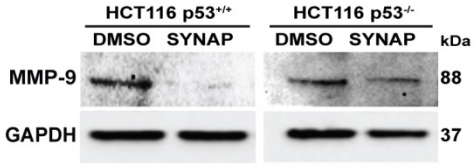
SYNAP was tested at 7 µM on HCT116 cells for 32h; Data are mean±SEM (n=5); *p<0.05

Chemotaxis cell migration assay



SYNAP was tested at 7 µM on HCT116 cells for 24h; Data are mean±SEM (n=5); *p<0.05

Western blot

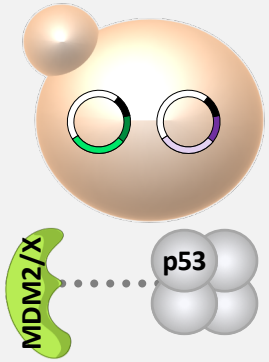


SYNAP was tested at 7 µM on HCT116 cells for 24h; Data are mean±SEM (n=3); *p<0.05

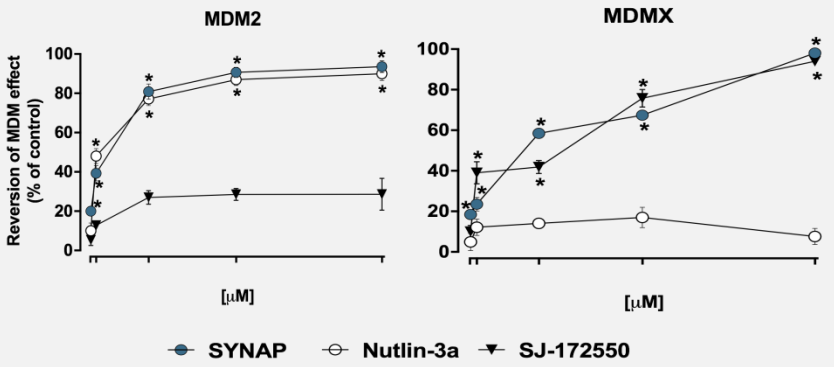
SYNAP activates p53 by inhibiting its interaction with MDM2 and MDMX

Soares et al., Eur J Pharm Sci, 2014, S0928-0987(14).

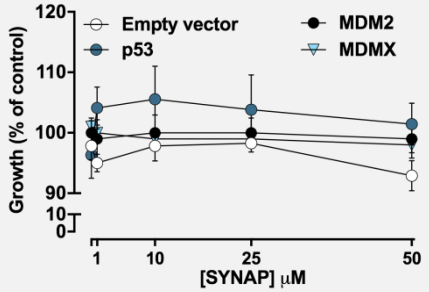
Saccharomyces cerevisiae



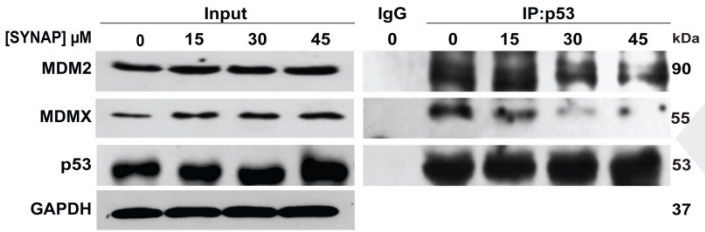
Yeast screening assay



Effect of 0.1-50 μM SYNAP, nutlin-3a and SJ-172550 on the reversion of MDM2/MDMX effect, by reestablishment of p53-induced growth inhibition, in yeast cells co-expressing human p53 and MDM2 or MDMX, after 42 h treatment; data are mean ± SEM (n=6); *P<0.05



Effect of 0.1–50 μM SYNAP on the growth of yeast cells expressing p53, MDM2 or MDMX alone, and yeast transformed with empty vectors, after 42 h treatment; data are mean ± SEM (n=6);*P<0.05.



Co-immunoprecipitation

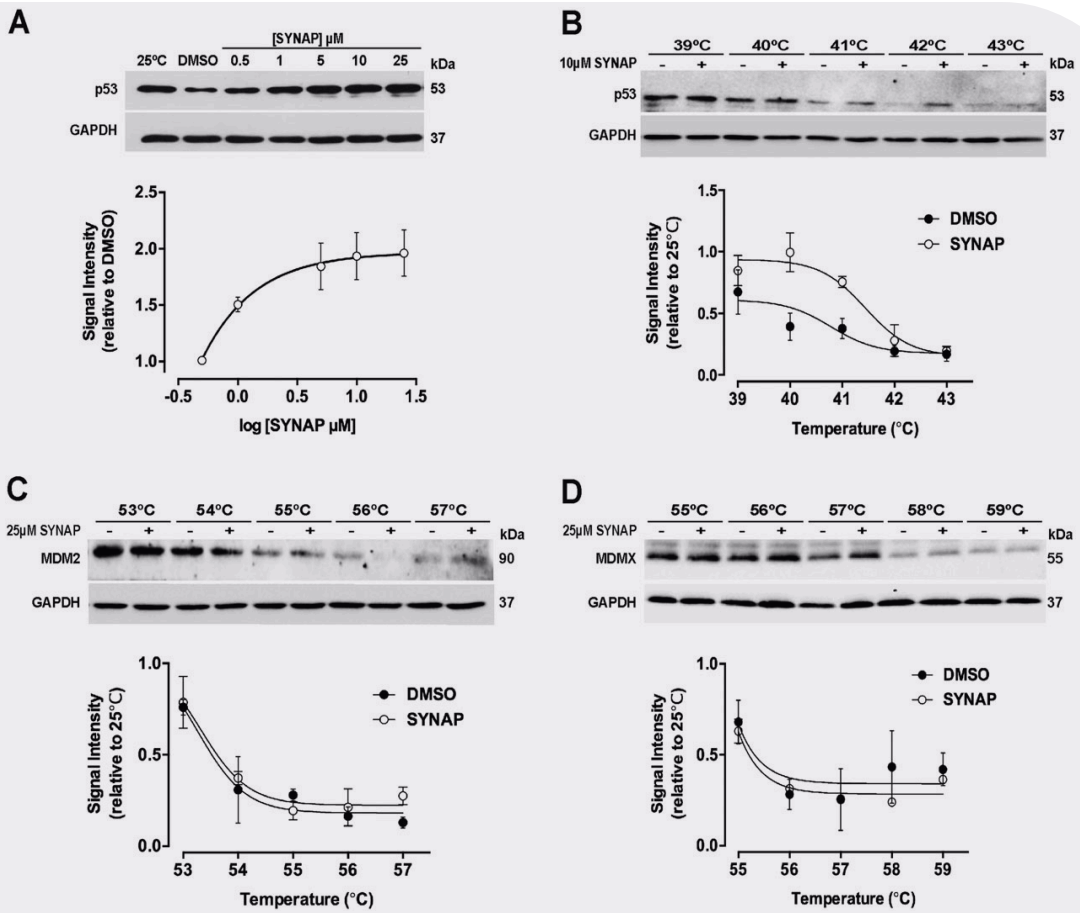
Human HCT116 tumor cells

SYNAP was tested at 15, 30 and 45μM on HCT116 p53$^{+/+}$ cells for 16h.

SYNAP induces p53 thermal stabilization in human colon cancer cells

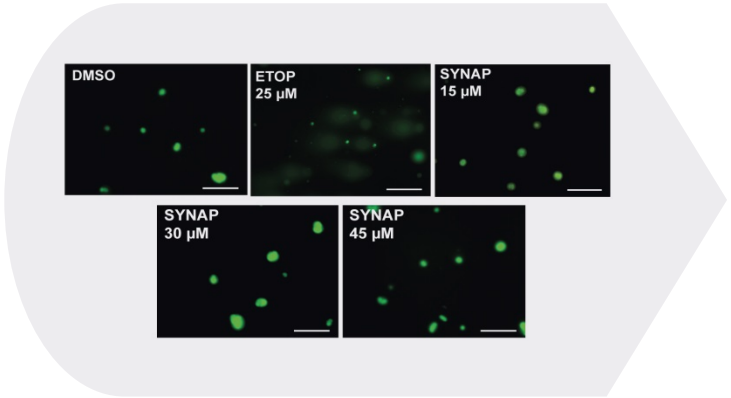
Cellular thermal shift assay (CETSA)

Human HCT116 tumor cells



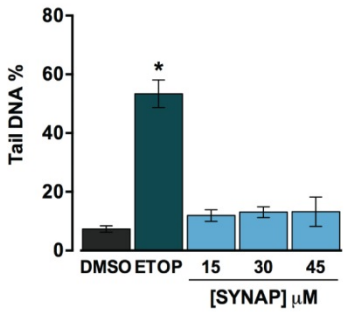
(A) Lysate samples treated with increasing concentrations of SYNAP were heated at 41°C; plot represents the increase of non-denatured p53 in SYNAP-treated samples relative to DMSO. (B–D) Lysate samples, obtained after treatment with SYNAP, were heated at different temperatures; plots represent the signal intensity of p53 (B), MDM2 (C) and MDMX (D) normalized to the signal intensity at 25°C.

SYNAP is non-genotoxic in human colon cancer cells and has low growth inhibitory effect against normal cells

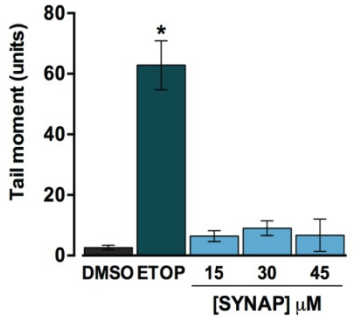


Measurement of DNA damage in HCT116 p53$^{+/+}$ cells with SYNAP, after 48 h treatment; data are mean ± SEM (n=5).

Comet assay

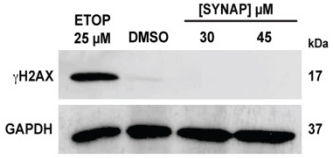


Quantification of tail DNA percentage; data are mean ± SEM, (n=5).



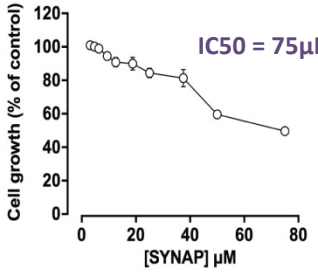
Quantification of tail moment; data are mean ± SEM, (n=5).

Histone phosphorylation



Analysis of YH2AX expression levels after 48h treatment with SYNAP.

Sulforhodamine B *assay in HFF-1 cells*



Concentration-response curves of SYNAP in HFF-1 normal human cells, after 48 h treatment; data are mean ± SEM, n=5.

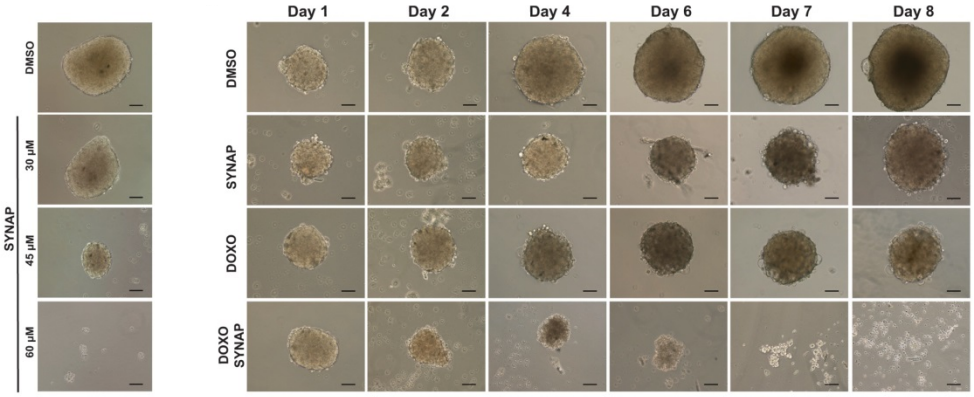
SYNAP sensitizes human colon cancer cells to the effect of conventional chemotherapeutic agents

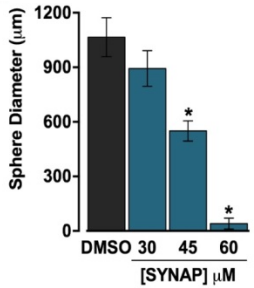
Drug combination using SRB assay

	Mutually nonexclusive CI		Dose reduction index (DRI)	
Drug combination with SYNAP	CI	Profile	SYNAP	Conventional Drug
DOXO (nM)				
18.7	0.676	Sinergy	2.113	4.926
37.5	0.762	Sinergy	2.432	2.847
75	0.701	Sinergy	3.762	2.247
150	0.616	Sinergy	6.902	2.121
Cisplatin (μM)				
0.5	0.801	Sinergy	1.623	5.397
1	0.832	Sinergy	1.836	3.467
2	0.987	Additive	1.997	2.056
4	1.08	Additive	2.408	1.053
5-FU (μM)				
0.65	0.889	Sinergy	1.812	2.957
1.25	0.839	Sinergy	2.194	2.606
2.5	0.833	Sinergy	2.649	2.192
5	0.762	Sinergy	3.378	2.142
ETOP (μM)				
0.38	1.099	Additive	1.287	2.577
0.75	0.867	Sinergy	1.834	3.110
1.5	0.803	Sinergy	2.300	2.711
3	1.099	Additive	2.298	1.351

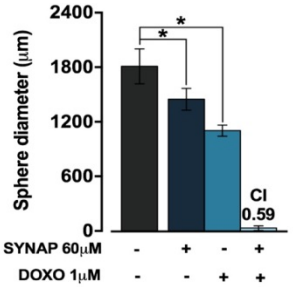
Effect of 7 μM SYNAP in combination with chemotherapeutic drugs, in HCT116 $p53^{+/+}$ cells, was evaluated using CompuSyn software to calculate combination index (CI) and dose reduction index (DRI) values for each combined treatment. CI<1, synergy; 1<CI<1.1, additive effect; CI>1.1, antagonism. Data were calculated using a mean value effect (n=6)

3D colon cancer spheroids





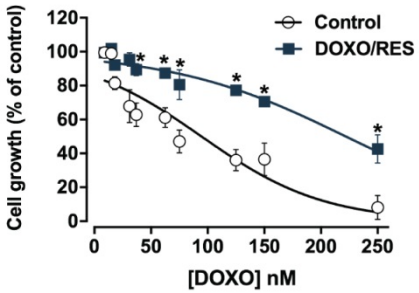
Spheroids formation in HCT116 $p53^{+/+}$ after 96 h treatment with SYNAP; Treatment performed at the seeding time. Determination of spheroids diameter at the end of treatment. Data are mean±SEM (n=5); *p<0.05



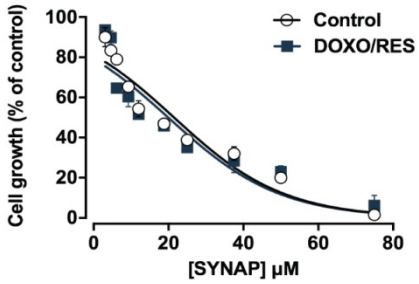
3-day-old HCT116 $p53^{-/-}$spheroids, treated with SYNAP for up to 8 days. Determination of spheroids diameter at the end of treatment; Data are mean±SEM (n=5); *P<0.05 . CI determined considering spheroid diameter.

Colon cancer cells develop resistance to DOXO but not to SYNAP: DOXO-resistant cancer cells show no cross-resistance to SYNAP and are re-sensitized to DOXO effect by SYNAP

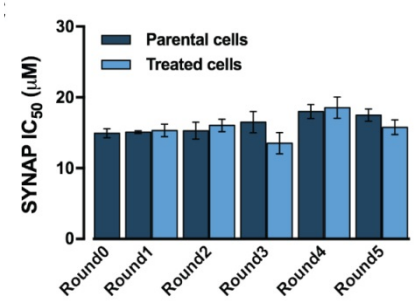
Sulforhodamine B *assay*



Concentration-response curves for DOXO in control and DOXO-resistant (DOXO/RES) HCT116 cells, after 48 h treatment. Data are mean±SEM (n=5); *p<0.05



Concentration-response curves for SYNAP in control and DOXO-resistant (DOXO/RES) HCT116 cells, after 48 h treatment. Data are mean±SEM (n=5); *p<0.05



IC50 values for SYNAP in 6 generations of cells (5 rounds of cells treated with 15, 30, 45, 60 and 75 µM SYNAP), after 48 h treatment. Data are mean±SEM (n=5).

Drug combination using SRB assay

	Mutually nonexclusive CI		Dose reduction index (DRI)	
Drug combination with SYNAP DOXO (nM)	CI	Profile	SYNAP	Conventional Drug
25	0.360	Sinergy	5.182	5.975
62.5	0.585	Sinergy	5.282	2.522
125	0.411	Sinergy	7.649	3.564
250	0.453	Sinergy	9.112	2.912

Effect of 7 µM SYNAP in combination with DOXO in DOXO/RES HCT116 cells, was evaluated using CompuSyn software to calculate combination index (CI) and dose reduction index (DRI) values for each combined treatment. CI<1, synergy; 1<CI<1.1, additive effect; CI>1.1, antagonism. Data were calculated using a mean value effect (n=6).

Conclusions

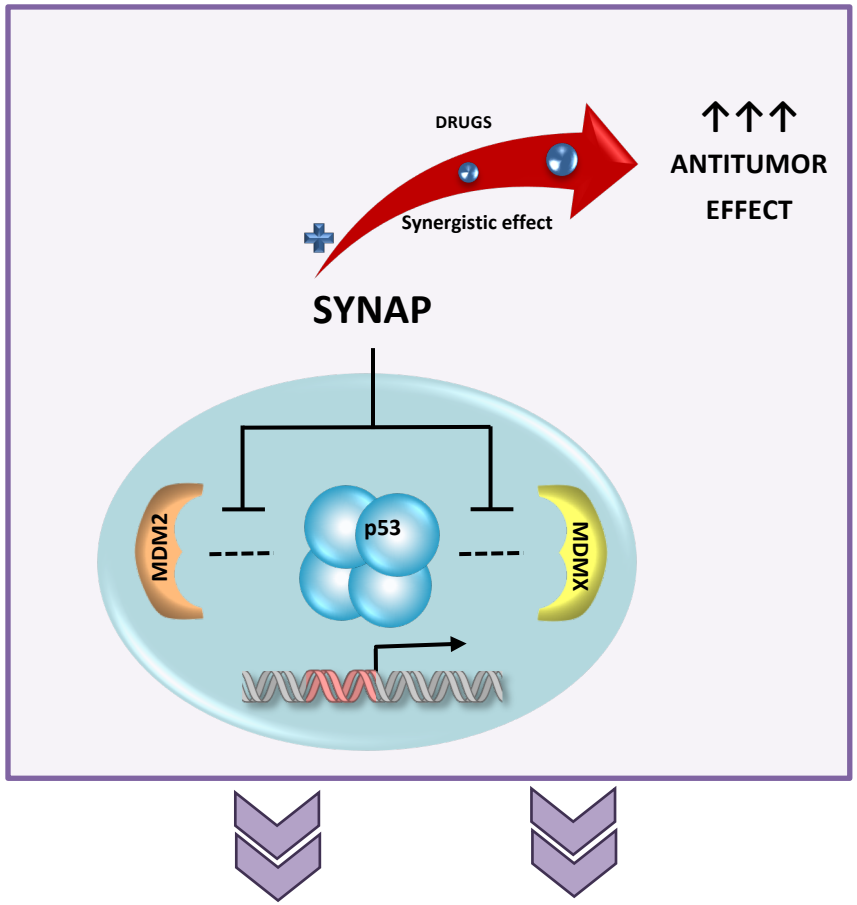
→ SYNAP is a new p53-activating agent

→ SYNAP activates p53 through disruption of the p53-MDM2/MDMX interactions and potential interaction with p53

→ SYNAP sensitized colon cancer cells to the cytotoxic effect of known chemotherapeutic agents

→ SYNAP did not present acquired or cross-resistance

→ SYNAP may represent the starting point for improved p53 activators



New encouraging anticancer drug candidate, alone or combined with conventional chemotherapeutics in precision therapy of colon cancer

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