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SLMP53-1 inhibits tumor cell growth through regulation of glucose metabolism and angiogenesis in a P53-dependent manner

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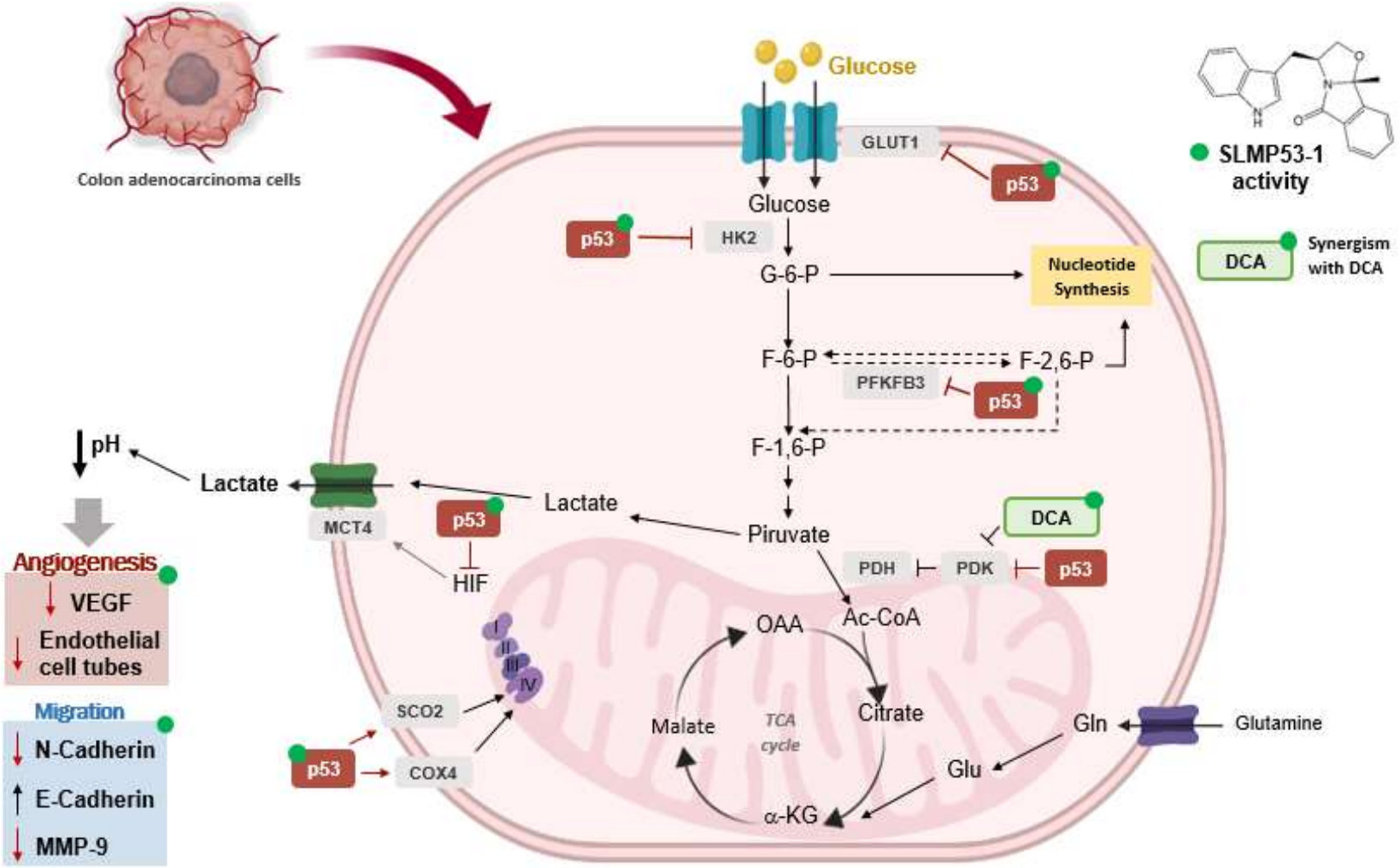


[3] Ramos *et al*. International Journal of Molecular Sciences.2020;21(2):596.

Abstract: The Warburg effect is an emerging hallmark of cancer, which has p53 as its major regulator. Recently, we have reported the (*S*)-tryptophanol-derived oxazoloisoindolinone (SLMP53-1) as a new p53-activating agent with *in vitro* and *in vivo* antitumor activity. Herein, we investigated the molecular events underlying the antitumor activity of SLMP53-1 in colon cancer by analyzing its effect on glucose metabolism, angiogenesis and migration.

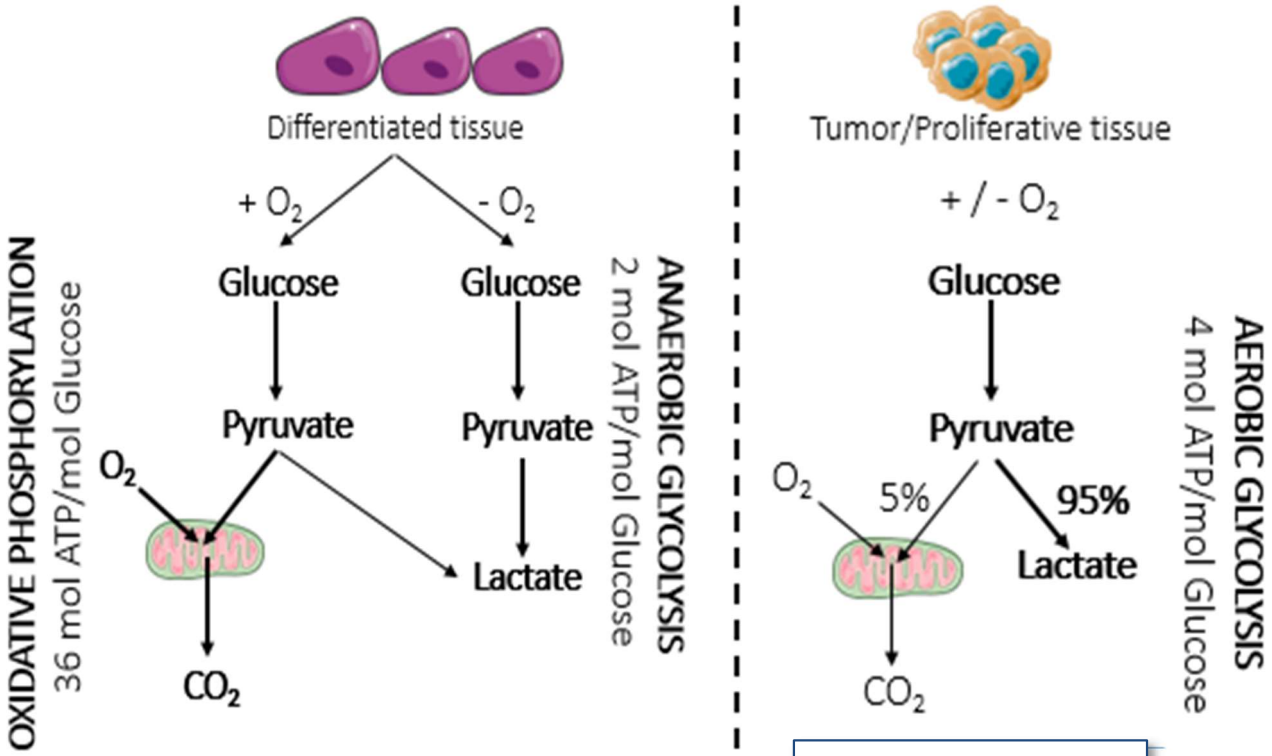
In colon HCT116 cancer cells, SLMP53-1 inhibited glycolysis through reduction of GLUT1, hexokinase-2, and phosphofructokinase-2 isoform 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 protein levels, also depleting the lactate export in colon cancer cells. Conversely, it enhanced mitochondrial oxidative phosphorylation (OXPHOS), upregulating the synthesis of cytochrome-c oxidase 2 and cytochrome-c oxidase subunit 4. SLMP53-1 further increased E-cadherin and reduced metalloproteinase-9 levels, which corroborated an inhibition of extracellular matrix (ECM) remodeling and epithelial-to-mesenchymal transition (EMT). Consistently, SLMP53-1 depleted angiogenesis, decreasing endothelial cell tube formation and vascular endothelial growth factor protein levels. In tumor tissues of xenograft mouse models carrying $p53^{+/+}$- and $p53^{-/-}$-HCT116 cells treated with SLMP53-1 or vehicle obtained in our previous work, the levels of molecular markers of glycolysis, OXPHOS, angiogenesis and migration were evaluated, confirming the *in vitro* results and unveiling that SLMP53-1 effect is p53-dependent in tumor tissues of colon cancer xenografts. SLMP53-1 exhibited synergistic cytotoxicity with the metabolic regulator dichloroacetic acid.

These data reinforce the promising application of SLMP53-1 in cancer therapy by targeting p53-mediated pathways of growth and dissemination.

Keywords: anticancer drug, anti-angiogenic, anti-migratory, glycolysis and OXPHOS, p53

Soares *et al*. Oncotarget. 2016;7(4):4326-4343;
Saraiva *et al*. European patent application nºEP3013833-A1, US-patent nº20160347765;
Ramos *et al*. International Journal of Molecular Sciences. 2020;21(2):596.

Warburg effect in tumor cells

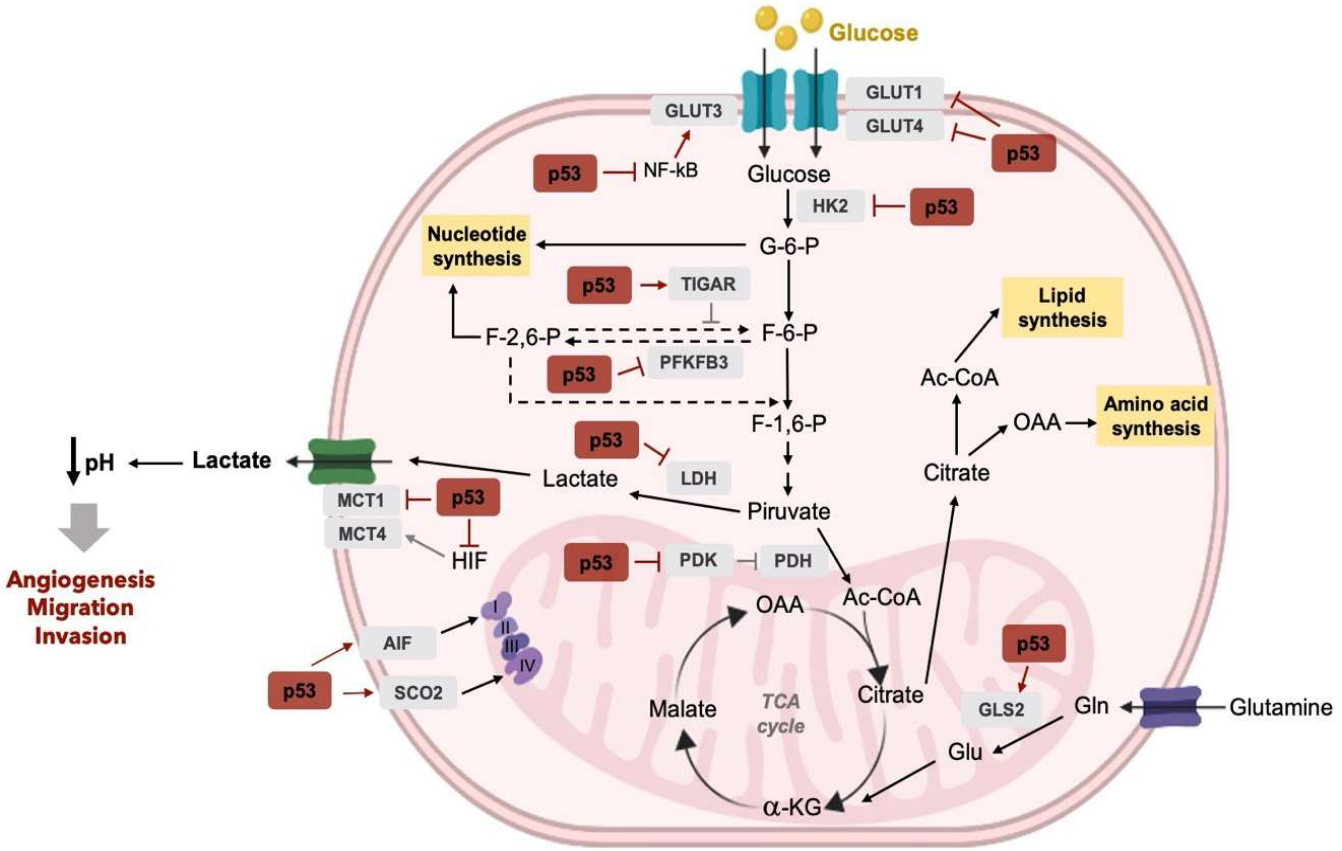


Warburg effect

Even in the presence of sufficient oxygen, most cancer cells increased glucose consumption and converted it to lactate, instead of relying on mitochondrial oxidative phosphorylation (OXPHOS)

A.S. Gomes *et al*. p53 and glucose metabolism: an orchestra to be directed in cancer therapy, Pharmacological Research. 131 (2018) 75–86.

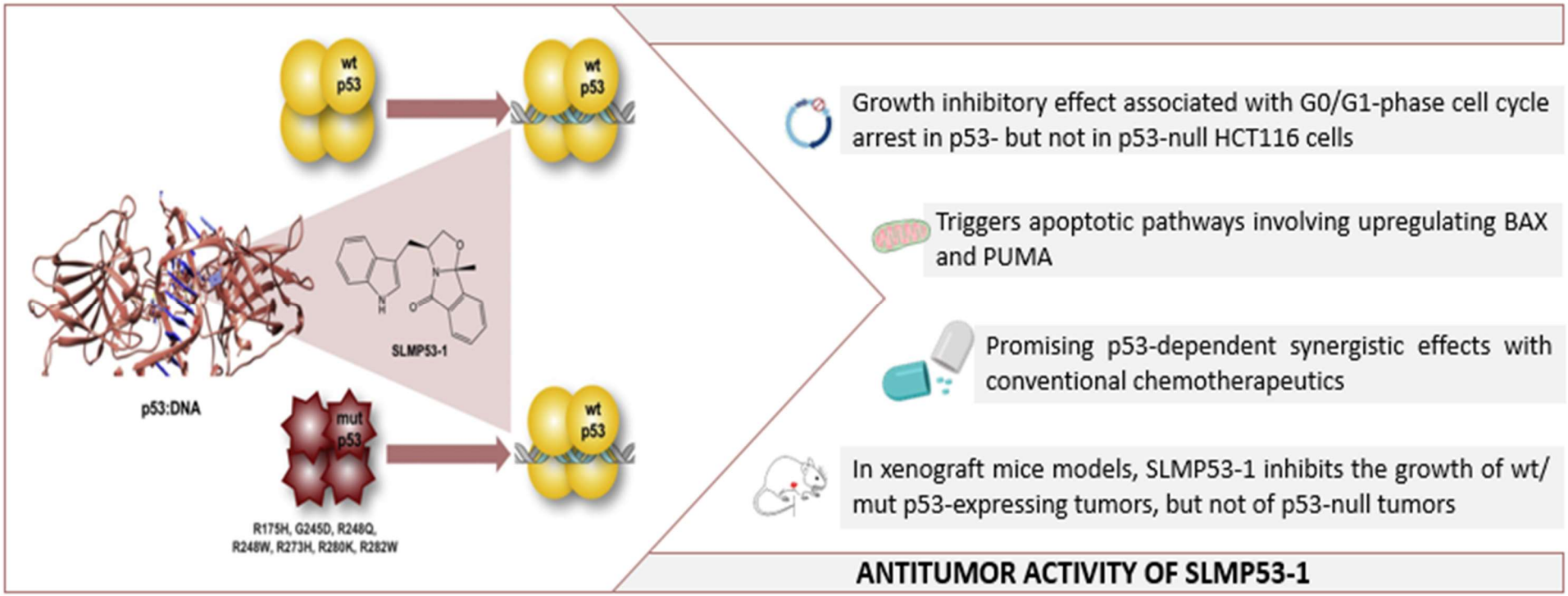
p53 as a regulator of metabolic reprogramming in tumor cells



p53 regulates important glucose metabolism mediators, with impact in maintaining mitochondrial health, increasing mitochondrial respiration and lowering glycolysis

A.S. Gomes *et al*. p53 and glucose metabolism: an orchestra to be directed in cancer therapy, Pharmacological Research. 131 (2018) 75–86.

(*S*)-tryptophanol-derived oxazoloisoindolinone (SLMP53-1) as a new activator of wt- and mutp53 with *in vitro* and *in vivo* antitumor activity



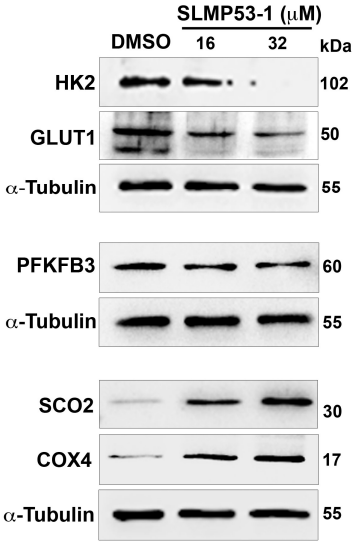
AIM

To investigate the molecular events underlying the antitumor activity of SLMP53-1 in colon cancer by analyzing its effect on glucose metabolism, angiogenesis and migration

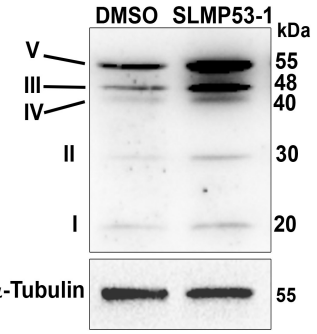
Soares *et al*. Oncotarget. 2016; 7(4):4326-4343.

Saraiva et al. European patent application nºEP3013833-A1, US-patent nº20160347765

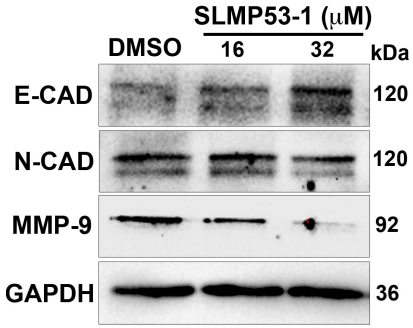
SLMP53-1 regulates the Warburg effect and angiogenesis in cancer cells, with interference in ECM remodeling and EMT events



Immunoblotting. HCT116 cancer cells treated for 24h (n=3).



Mitochondrial complexes expression by immunoblotting. HCT116 cells treated with 16μM SLMP53-1 for 24h (n=3).



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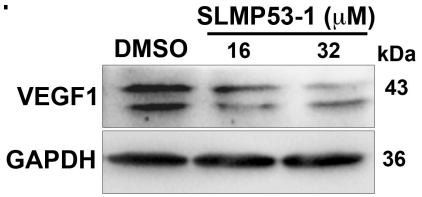
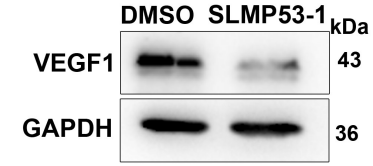
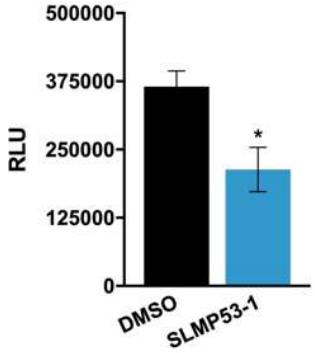


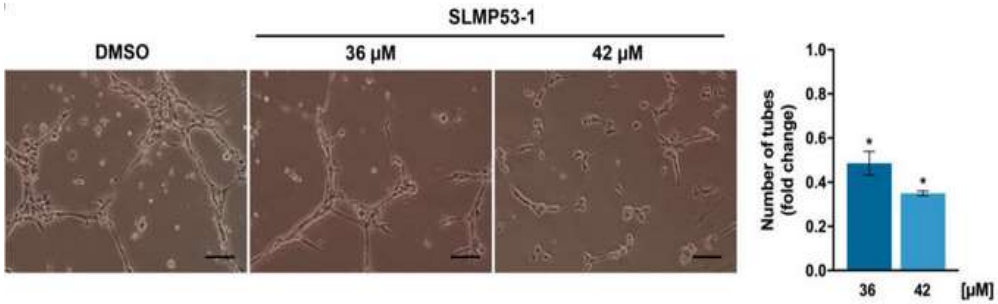
Immunoblotting. HCT116 cells treated for 24h (n=3).



Immunoblotting. HMVEC-D endothelial cells treated with 42μM SLMP53-1 for 48h (n=3).



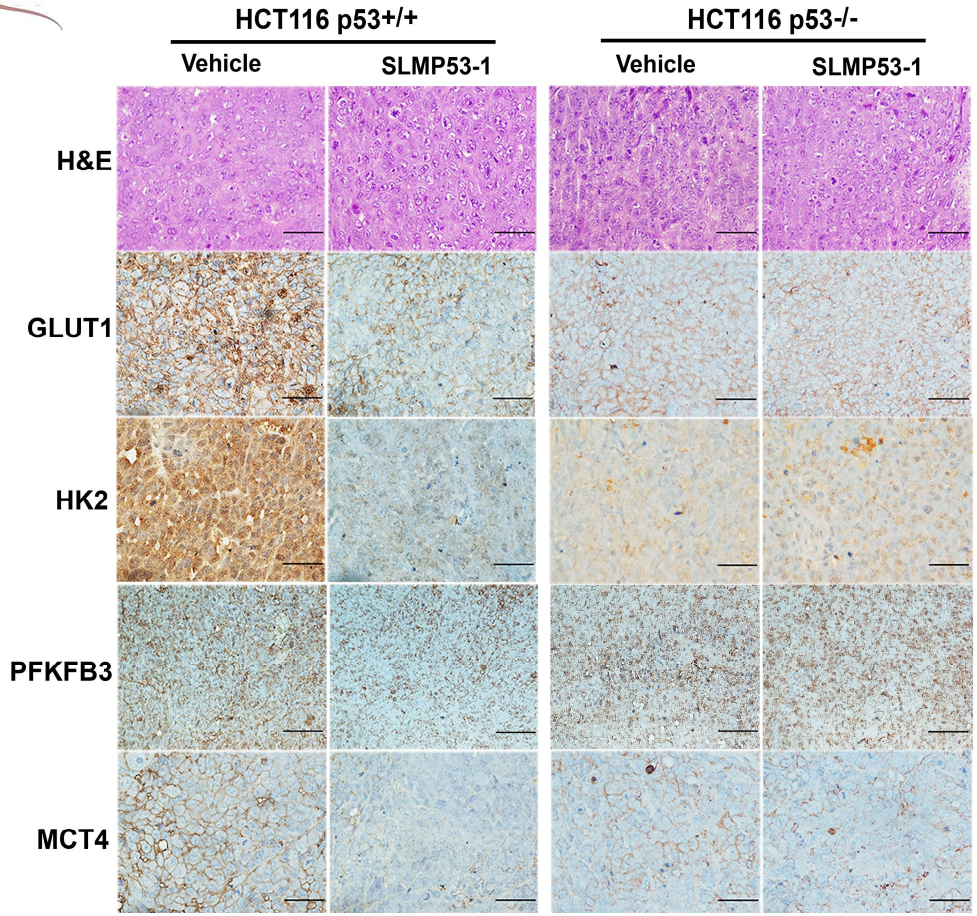
Lactate secretion assay. HCT116 cells treated with 16μM SLMP53-1 or DMSO for 24h; Relative luminescence corresponds to mean ± SEM (n=3). Values significantly different from DMSO: * $p < 0.05$, unpaired Student's t-test).



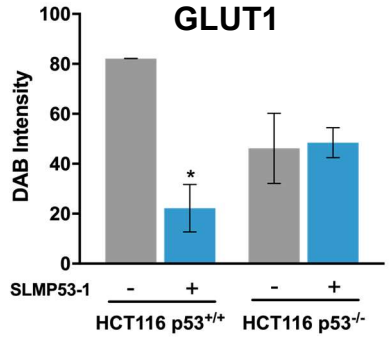
Endothelial cell tube formation assay. Tube-like structures of HMVEC-D cells treated for 12h; (5 randomly selected microscopic fields); quantification represented by mean ± SEM (n=3). * $p < 0.05$; one-way ANOVA with Dunnett's multiple comparison test.

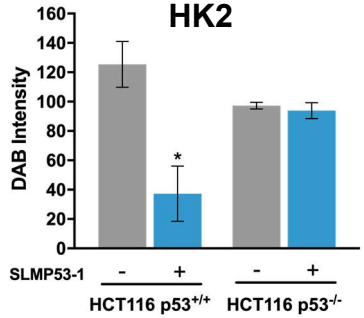
SLMP53-1 regulates the Warburg effect and angiogenesis, interfering with ECM remodeling and EMT events in a p53-dependent manner, in tumor tissue of xenograft mouse models

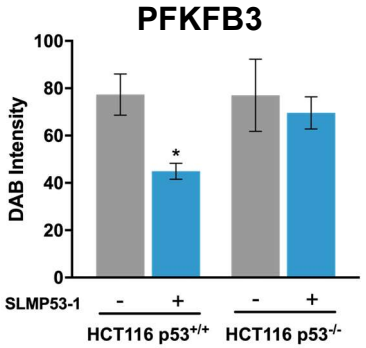
Balb/c nude mice

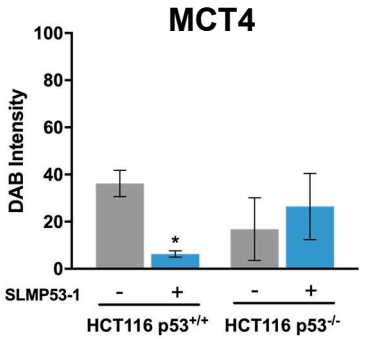


GLUT1 ↓
HK2 ↓
PFKFB3 ↓
MCT4 ↓
in p53 $^{+/+}$, but not in p53 $^{-/-}$ HCT116 tumors





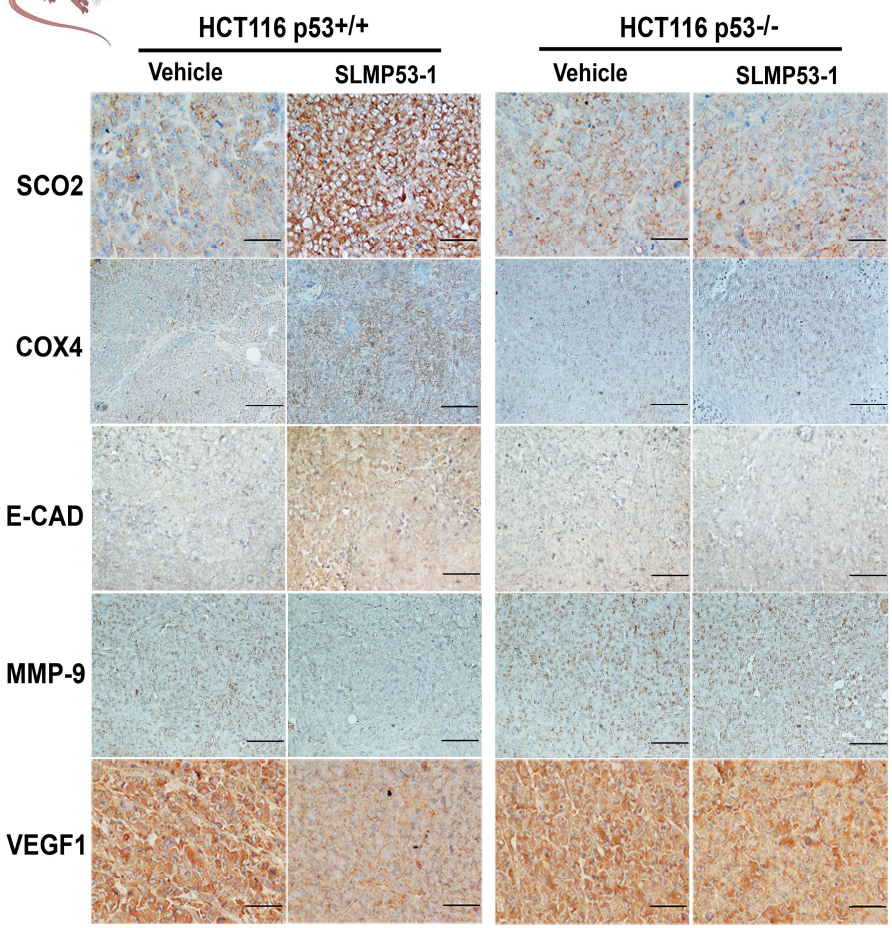


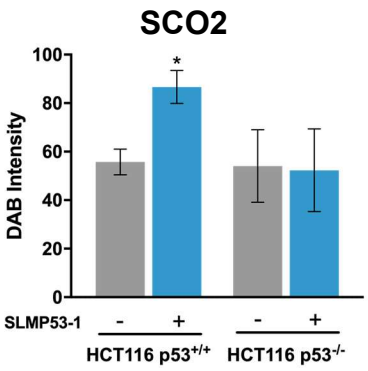


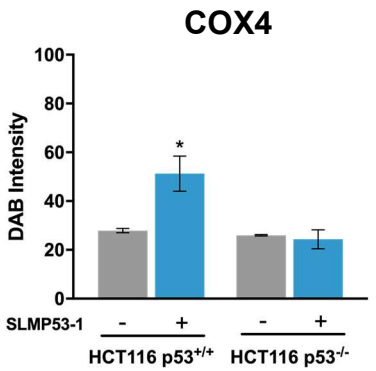
Imunohistochemistry. Tumor tissues xenografts treated with 50 mg/kg SLMP53-1 or vehicle (IP injection 2x/week, 5 administrations obtained from Soares *et al*, Oncotarget (2016); Scale bar = 20 µm; magnification = x200. Quantification of IHC staining in tumor tissues treated with SLMP53-1 (+) or vehicle (-). Data are mean ± SEM (n=3); * $p < 0.05$, unpaired Student's *t*-test.

SLMP53-1 regulates the Warburg effect and angiogenesis, interfering with ECM remodeling and EMT events in tumor tissue of xenograft mouse models

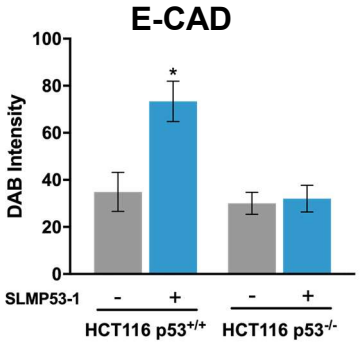
Balb/c nude mice

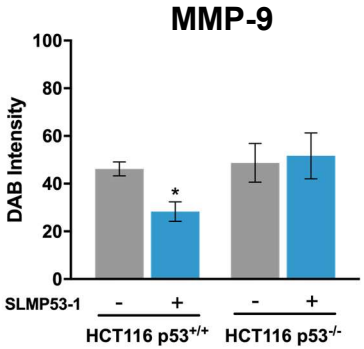


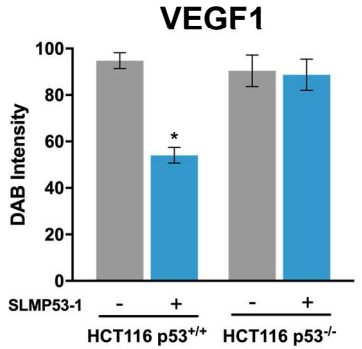




SCO2 ↑
COX4 ↑
E-CAD ↑
MMP-9 ↓
VEGF1 ↓
in p53 $^{+/+}$, but not in p53 $^{-/-}$
HCT116 tumors







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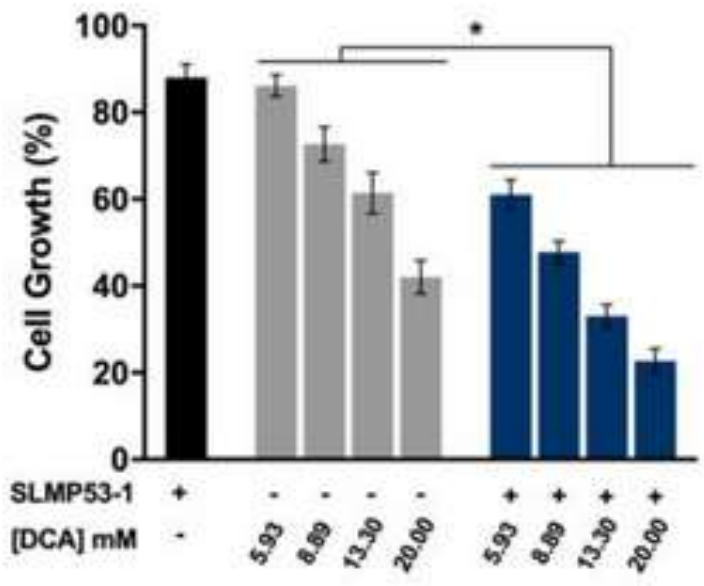
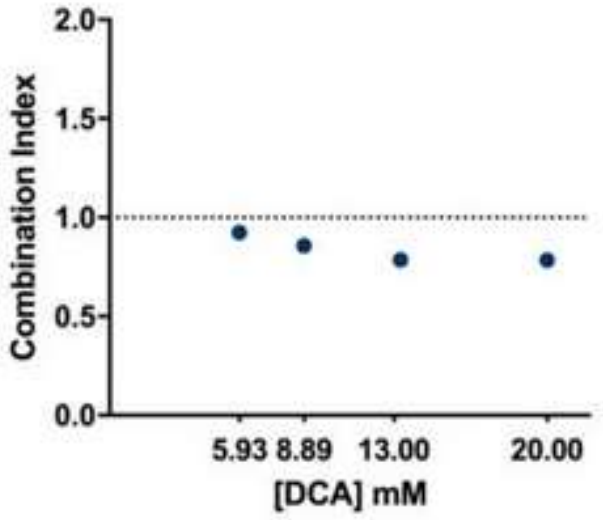
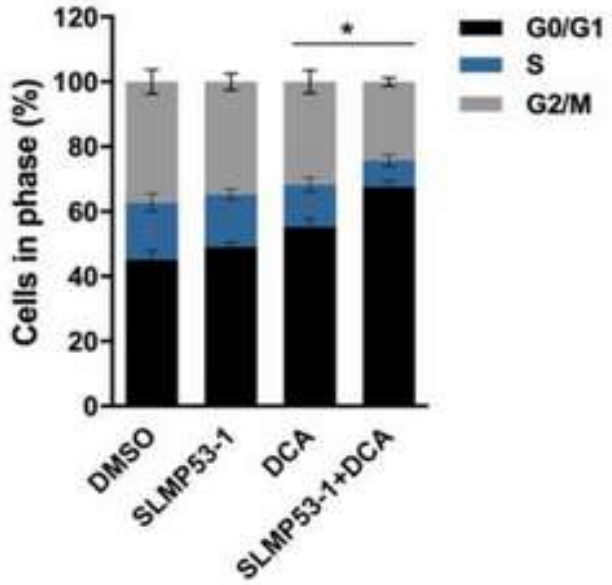
SLMP53-1 synergizes with dichloroacetic acid (DCA), enhancing its antitumor efficacy in cancer cells



Sulforhodamine B assay. HCT116 cells treated with DCA alone and in combination with SLMP53-1 for 48h; growth obtained with solvent (DMSO) was set as 100%. Data are mean ± SEM (n=6); values different from DCA alone: * $p < 0.05$, two-way ANOVA followed by Sidak's test.



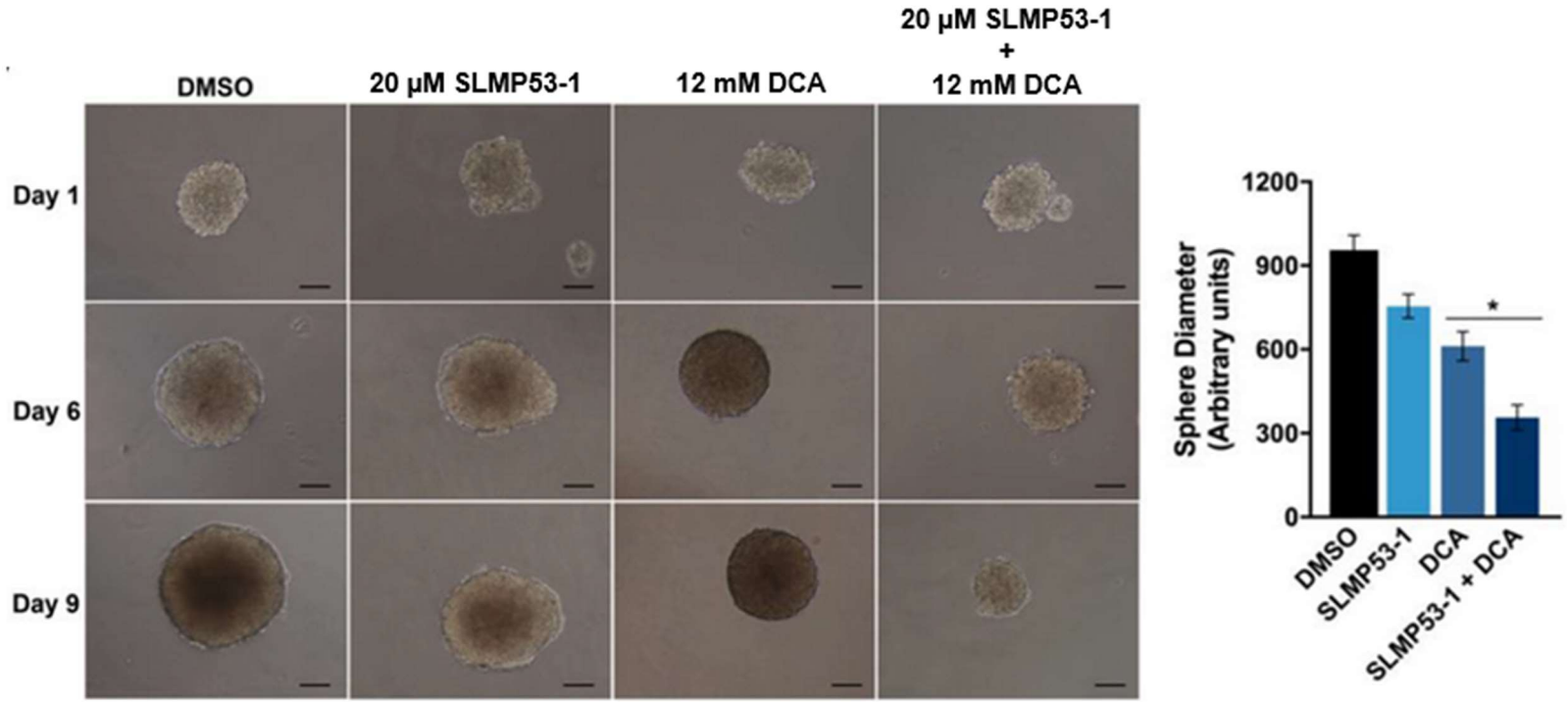
Combination therapy assay. CI values were calculated by using a mean value effect. CI < 1: synergy (n=6); CompuSyn software.



Cell Cycle analysis. HCT116 cells treated with DCA alone and in combination with SLMP53-1 for 48h; Data are mean ± SEM (n=3); Values significantly different from DCA alone: * $p < 0.05$, two-way ANOVA followed by Dunnett's test).

Synergistic effect of SLMP53-1 with the metabolic regulator DCA, with increase of G0/G1-phase cell cycle arrest

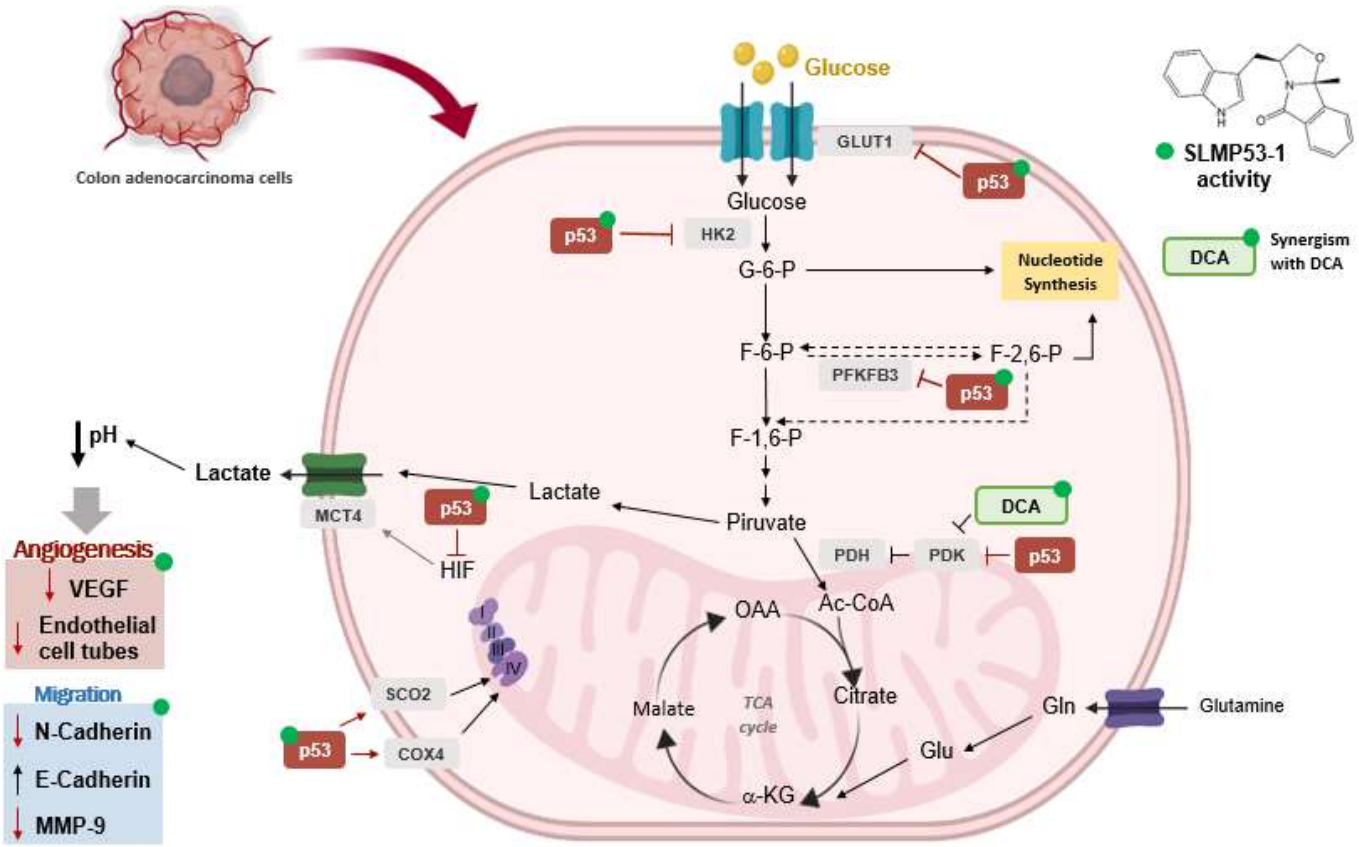
SLMP53-1 synergizes with dichloroacetic acid (DCA), enhancing its antitumor efficacy in cancer cells



HCT116 colonospheres. Cells were treated with 20 μM SLMP53-1 alone and in combination with 12 mM DCA, for up to nine days of treatment (n=3); scale bar = 50 μm; magnification = ×100. Spheroid diameter data are mean ± SEM (n=3); values significantly different from DCA alone: * $p < 0.05$, one-way ANOVA followed by Dunnett's test.

SLMP53-1 was shown to synergize with DCA both in 2D and 3D cancer cell models

Conclusions



Potential promising antitumor activity of SLMP53-1, as single or combined anticancer drug, by targeting major pathways of cancer metabolism and dissemination

Acknowledgments

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through grants UID/QUI/50006/2020, UID/DTP/04138/2019, PTDC/QUI-QOR/29664/2017; PhD grants of PD/BD/143126/2019 (V. Barcherini), and 2020.04613.BD (J. Calheiros).

The authors declare the following competing financial interest: one patent application protecting the compound disclosed in this manuscript has been filed by the authors M.M.M Santos and L. Saraiva