

p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase

p53 通过直接抑制葡萄糖-6-磷酸脱氢酶活性调控细胞生物合成

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p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase

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Abstract

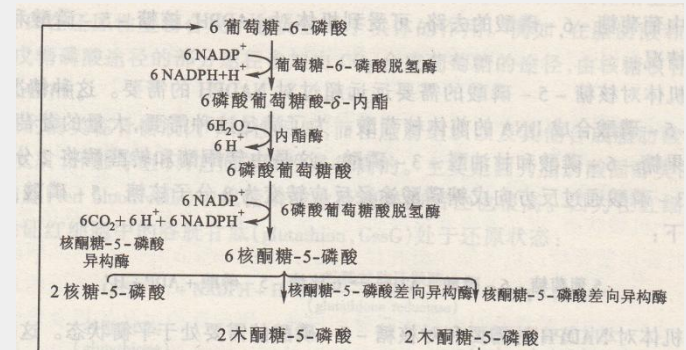
- ⊙ **Cancer cells consume large quantities of glucose and primarily use glycolysis for ATP production, even in the presence of adequate oxygen. This metabolic signature (aerobic glycolysis or the Warburg effect) enables cancer cells to direct glucose to biosynthesis, supporting their rapid growth and proliferation.**
- ⊙ 癌细胞生长需要消耗大量葡萄糖，主要是通过糖酵解产生ATP，但这种糖酵解甚至在氧充足条件下依然很活跃。有氧酵解或瓦博格效应的这种代谢特征促进了癌细胞直接将葡萄糖进行生物合成，维持癌细胞快速、无限增殖。

- 糖代谢有2种途径：线粒体氧化磷酸化和糖酵解。正常哺乳动物细胞在有氧条件下，糖酵解被抑制。然而，1920年，德国生物化学家Warburg发现：肝癌细胞的糖酵解活性较正常肝细胞活跃。提出：在氧气充足下，恶性肿瘤细胞糖酵解同样活跃，这种有氧糖酵解的代谢特征称为瓦博格效应，表现为葡萄糖摄取率高，糖酵解活跃，代谢产物乳酸含量高。
- **“瓦博格效应” 疑问—— “为什么肿瘤细胞大量消耗葡萄糖却不能高效产能？”**

- ⊙ **However, both causes of the Warburg effect and its connection to biosynthesis are not well understood. Here we show that the tumour suppressor p53, the most frequently mutated gene in human tumours, inhibits the pentose phosphate pathway (PPP).**
- ⊙ 然而，瓦博格效应产生的原因以及它与癌细胞生物合成的关系，还不是十分清楚。在本文中，介绍了一种抑癌基因p53（在肿瘤细胞中发生变异频率较高的基因），p53可抑制戊糖磷酸途径（pentose phosphate pathway，PPP）。

- Through the PPP, p53 suppresses glucose consumption, NADPH production and biosynthesis. The p53 protein binds to glucose-6-phosphate dehydrogenase (G6PD), the \perp first and rate-limiting enzyme of the PPP, and prevents the formation of the active dimer.

- 通过PPP，p53可抑制葡萄糖消耗、NADPH产生及生物合成。p53可以与戊糖磷酸途径上的第一步反应的关键酶葡萄糖-6-磷酸脱氢酶（G6PD）相结合，并抑制其活性（活性二聚体形成）。



- ⊙ **Tumour-associated p53 mutants lack the G6PD-inhibitory activity. Therefore, enhanced PPP glucose flux due to p53 inactivation may increase glucose consumption and direct glucose towards biosynthesis in tumour cells.**

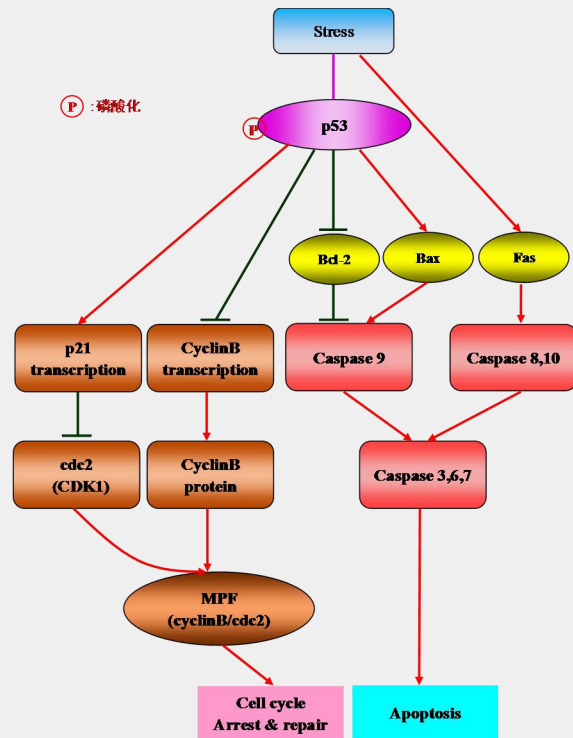
在肿瘤细胞内，由于p53发生突变，无法抑制G6PD活性，因此，由于被p53抑制的磷酸戊糖途径被激活，大量葡萄糖被消耗，而进行生物合成。

引言

- ⊙ **The tumour suppressor p53 invokes anti-proliferative processes, of which the best understood include cell cycle arrest, DNA repair and apoptosis.**

抑癌基因p53主要抑制癌细胞增殖过程，包括细胞周期停滞、DNA修复以及细胞凋亡等已研究比较清楚。

- p53可使周期蛋白*cyclinB*启动子区关闭而下调*cyclinB*的转录水平。如在卵巢癌细胞中，球毛壳甲素K在p53介导作用下，发生细胞G2期阻滞(Li *et al.*, 2015)。
- DNA损伤若被修复，细胞周期恢复正常；如果损伤严重，DNA无法被修复，细胞则经历凋亡。细胞凋亡的起始阶段的特征是Caspase被激活，主要有：**p53介导的线粒体凋亡通路**，**死亡受体凋亡通路**。



- ⊙ **Recent studies indicated that p53 also has a role in modulating metabolism including glycolysis and oxidative phosphorylation.**

近年来研究表明，p53在调节代谢方面有重要作用，包括葡萄糖酵解和氧化磷酸化。

- ⊙ **However, the role of p53 in regulating biosynthesis is less well understood.**

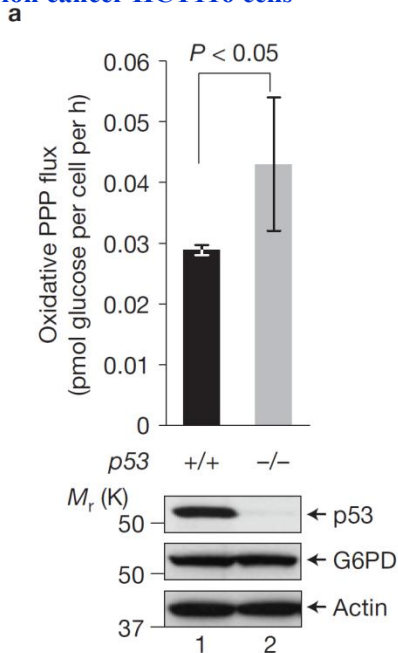
但是，关于p53在生物合成中的调节功能研究甚少。

- ⊙ **The PPP is important for both glucose catabolism and biosynthesis. In an oxidative phase, the PPP generates NADPH (nicotinamide adenine dinucleotide phosphate, reduced), the principal intracellular reductant required for reductive biosynthesis such as the synthesis of lipid, and ribose 5-phosphate, an essential precursor for biosynthesis of nucleotides.**
- ⊙ **This is followed by a non-oxidative interconversion of ribose 5-phosphate to the intermediates in the glycolytic pathways.**
- ⊙ **Despite the vital role of the PPP in biosynthesis and its close link to glycolysis, the regulation of the PPP in tumour cells remains unclear.**

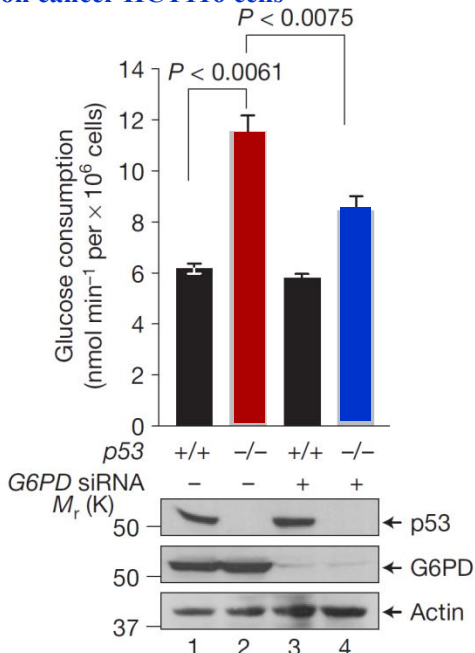
- ⊙ To investigate **whether** p53 modulates the PPP, we compared the oxidative PPP flux in isogenic $p53^{+/+}$ and $p53^{-/-}$ human colon cancer HCT116 cells. Cells were cultured in medium containing [2- ^{13}C]glucose, and the glucose metabolites were measured by nuclear magnetic resonance (NMR) spectroscopy.

p53 deficiency correlates with increases in PPP flux, glucose consumption and lactate production

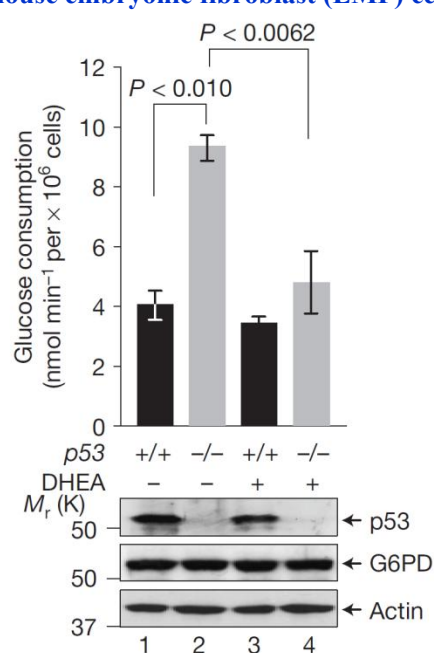
colon cancer HCT116 cells



colon cancer HCT116 cells

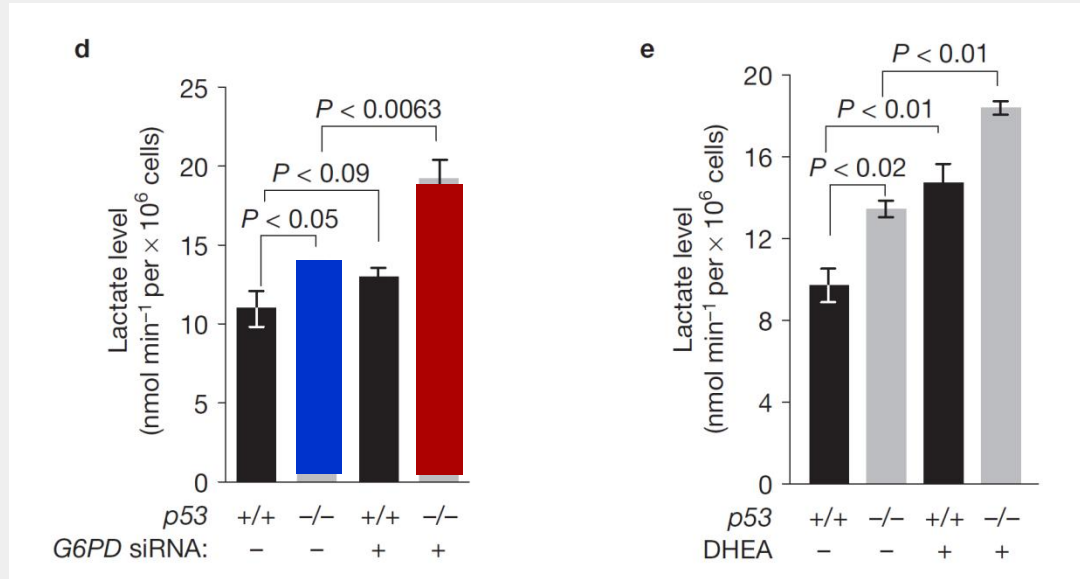


mouse embryonic fibroblast (EMF) cells



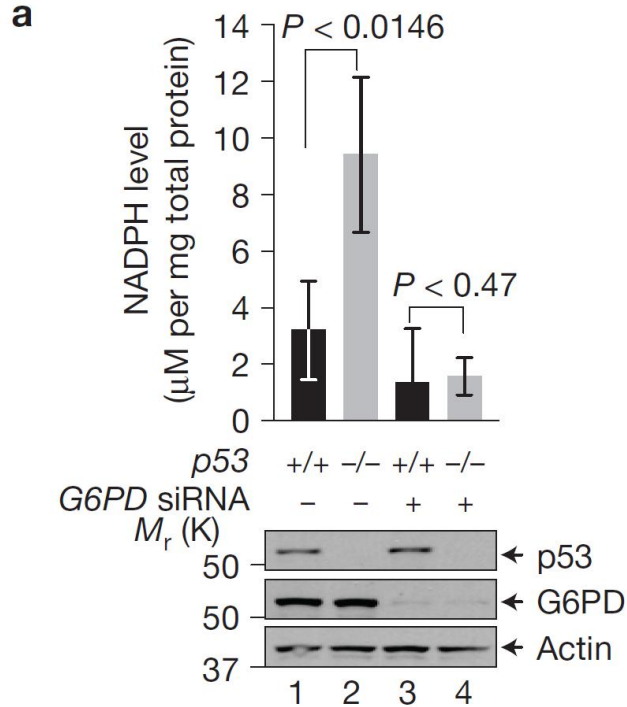
These results indicate that *p53* deficiency increases glucose consumption mainly through an enhanced PPP flux.

p53 deficiency correlates with increases in PPP flux, glucose consumption and lactate production

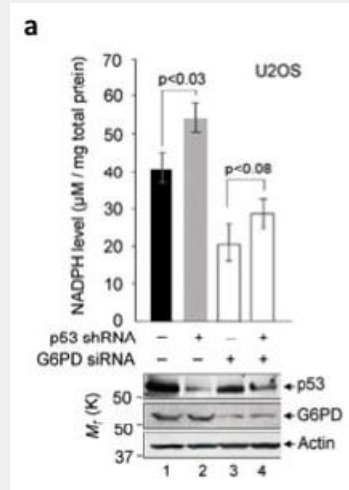


Inhibition of G6PD in these cells increased, rather than decreased, lactate production, regardless of *p53* status.

p53 regulates NADPH levels



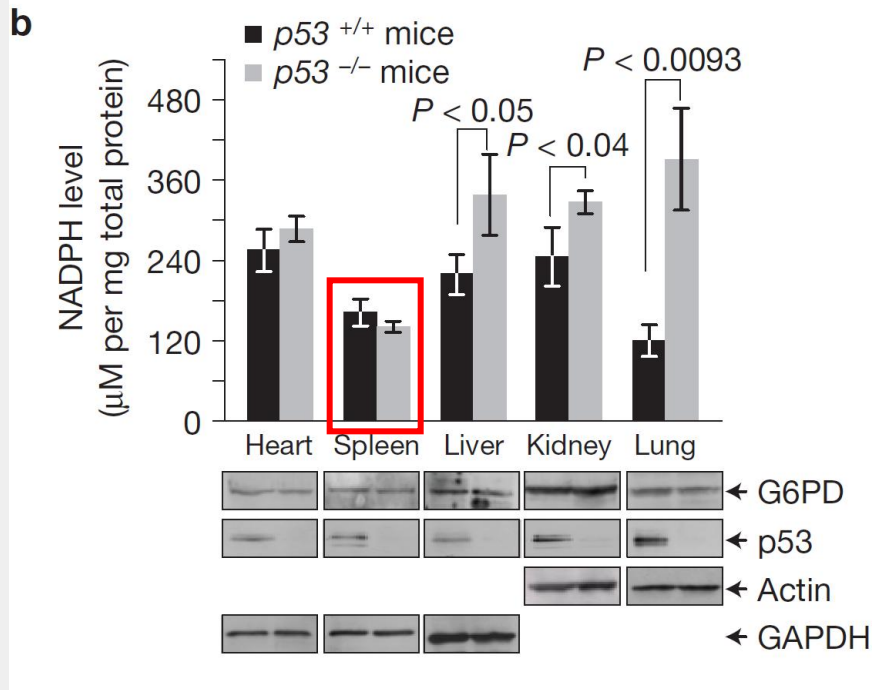
The PPP plays a significant role in the production of cellular NADPH. The lack of p53 led to a strong increase in the NADPH level in HCT116 cells.



Similarly, knocking down p53 in U2OS cells with small hairpin RNA (shRNA) strongly increased NADPH levels.

Figure 2 p53 regulates NADPH levels

p53 regulates NADPH levels

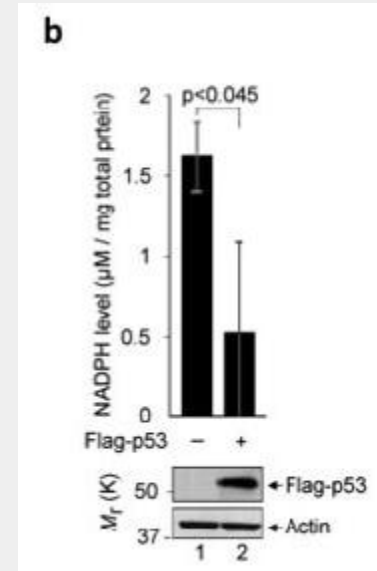
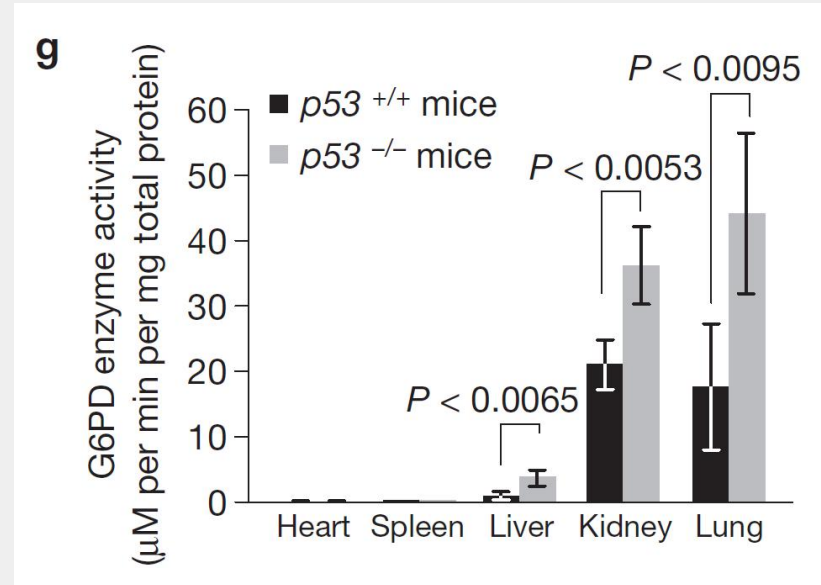


Treatment with G6PD siRNA minimized the difference in NADPH levels between $p53$ -proficient and -deficient cells.

The tissues from $p53^{-/-}$ mice—including heart, liver, kidney and lung—exhibited substantially elevated NADPH levels, compared with those in the corresponding tissues from $p53^{+/+}$ mice.

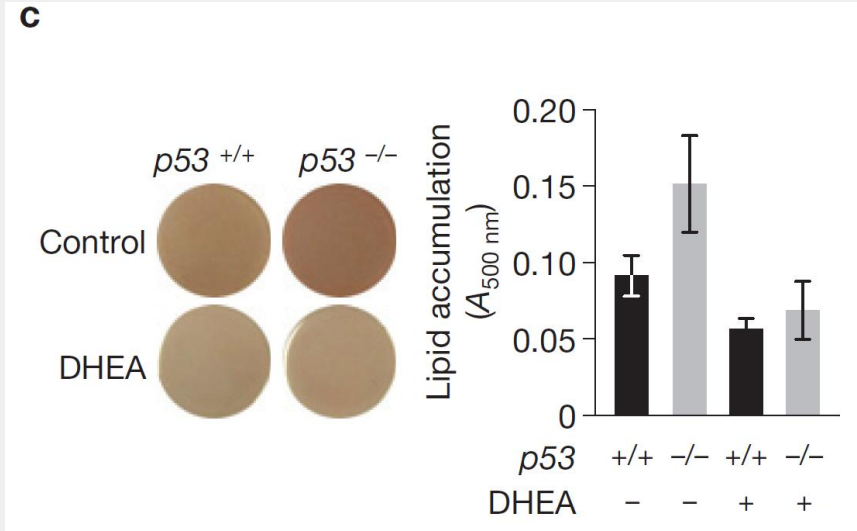
The **exception** was found in the **spleen**.

In the spleen, the activity of G6PD was very low (Fig. 2g), and the PPP might not contribute substantially to the overall NADPH production.



In contrast to p53 downregulation, overexpression of p53 led to a strong decrease in NADPH levels (Supplementary Fig. S1b).

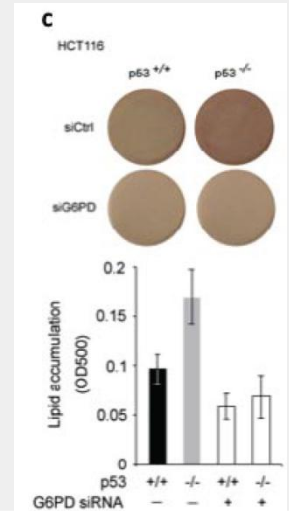
NADPH is required for the biosynthesis of lipid



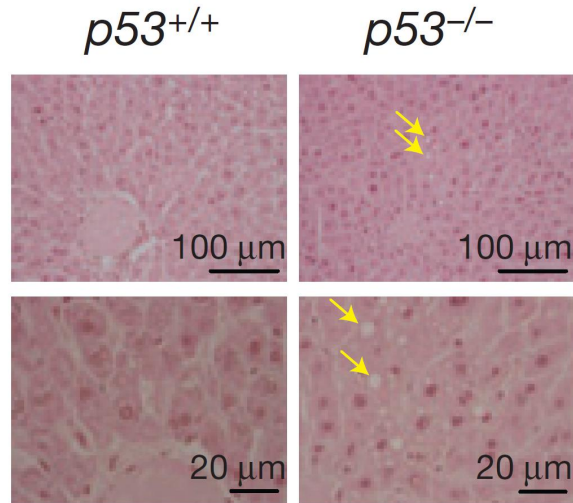
The $p53^{-/-}$ MEF cells showed enhanced lipid levels, compared with $p53^{+/+}$ MEF cells, as evaluated by Oil Red O staining.

The lack of p53 also resulted in higher levels of lipid in HCT116 cells.

The difference in lipid accumulation between $p53^{+/+}$ and $p53^{-/-}$ cells diminished on treatment with G6PD siRNA or DHEA.



d



We also evaluated the effect of p53 on the formation of fat droplets in the liver. The liver of $p53^{-/-}$ mice had **a larger number of bigger fat droplets**, compared with the liver of $p53^{+/+}$ mice.

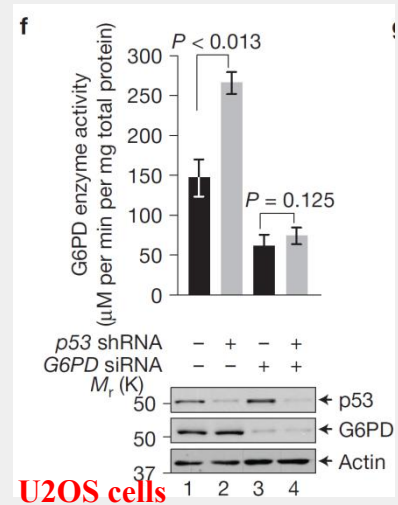
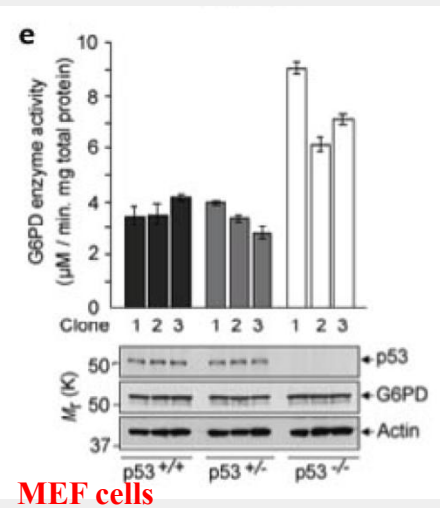
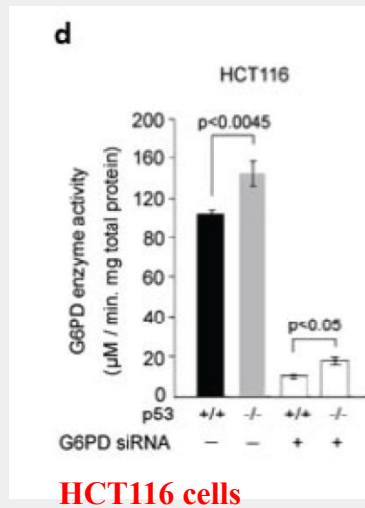
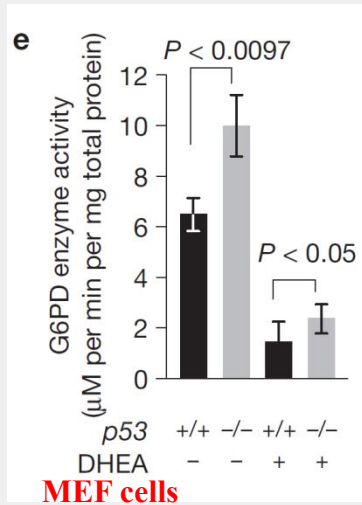
Histological sections of liver tissue from $p53^{-/-}$ and $p53^{+/+}$ mice were stained with haematoxylin and eosin. Arrows indicate fat droplets.

Together, these results indicate that **p53 inhibits NADPH production and lipid accumulation** by lowering the glucose flux through the PPP.

To investigate the **mechanism** by which p53 regulates the PPP, we assayed the **activity of G6PD**, a key regulatory point of the PPP.

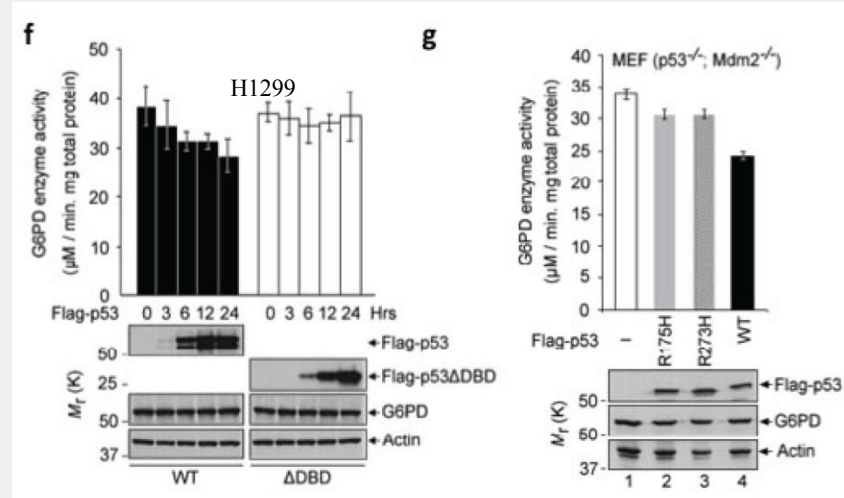
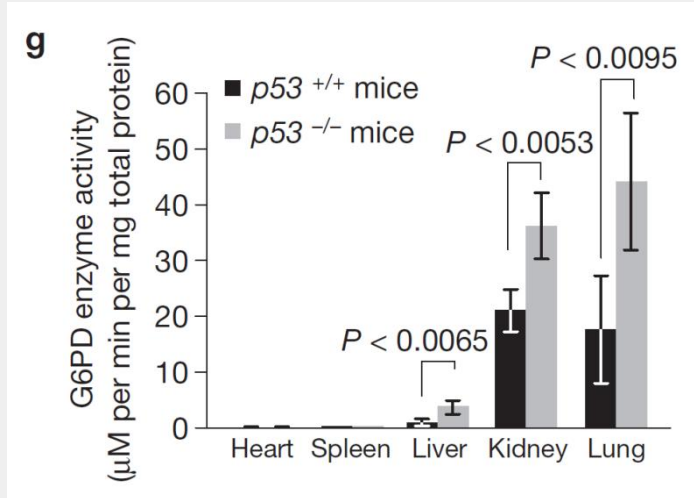
The lack of p53 correlated with a strong elevation in G6PD activity in both MEF and HCT116 cells.

Similarly, when p53 was knocked down in U2OS cells with shRNA, G6PD activity nearly doubled.



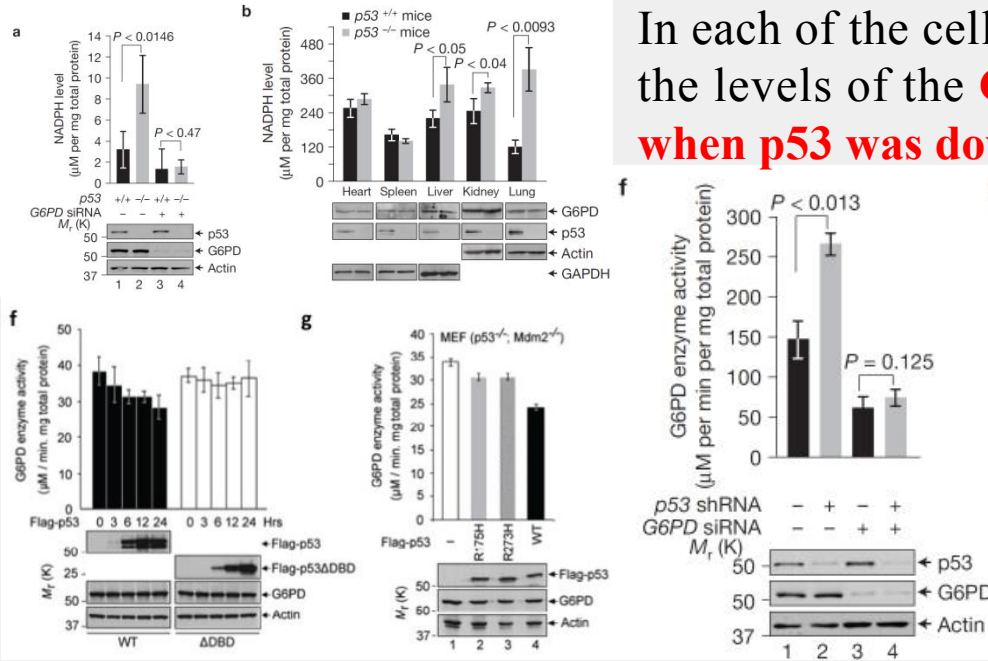
The **lack** of p53 was associated with **highly** elevated G6PD **activity**.

Conversely, **overexpression** of wild-type p53 in the p53-deficient cell lines (H1299 and p53^{-/-} Mdm^{-/-} MEF) caused a noticeable **decrease** in G6PD **activity**.

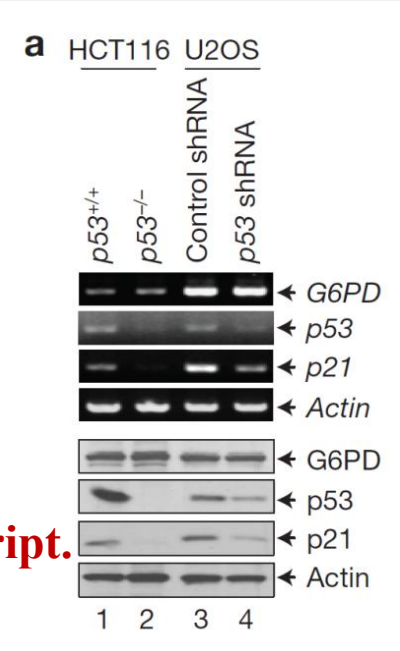


These results show that p53 suppresses G6PD activity.

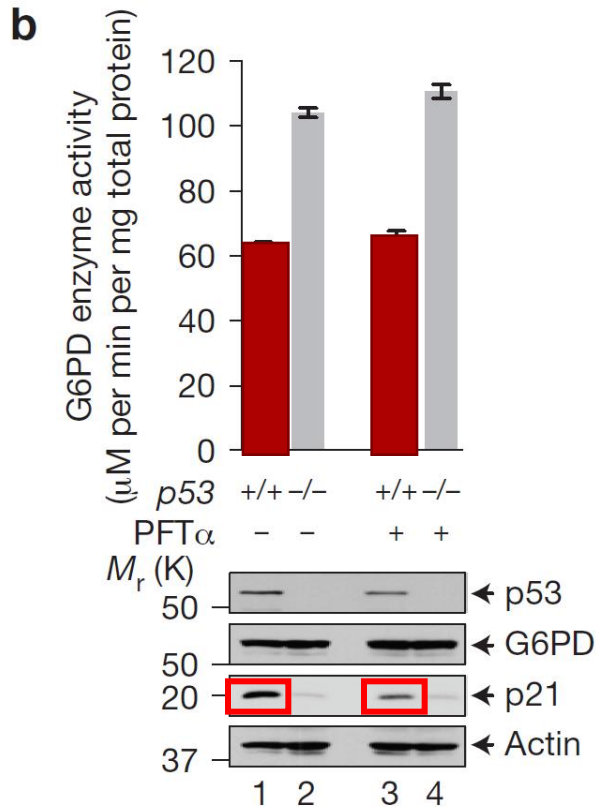
In each of the cell lines and tissues that were examined, the levels of the **G6PD protein remained unchanged when p53 was downregulated or overexpressed.**



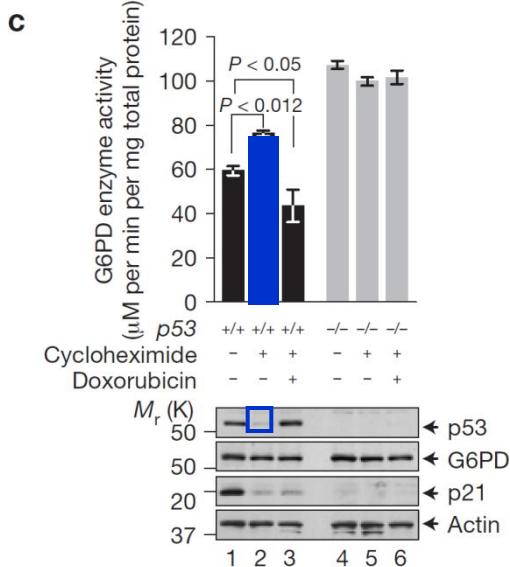
Moreover, p53 did not change the level of G6PD transcript.



To **rule out** the involvement of **other p53 target genes** in the inhibition of **G6PD**, we used an inhibitor of p53 transcriptional activity, pifithrin- α (PFT α).



PFT α impeded p53-induced expression of p21, but did not restore p53-inhibited G6PD activity.

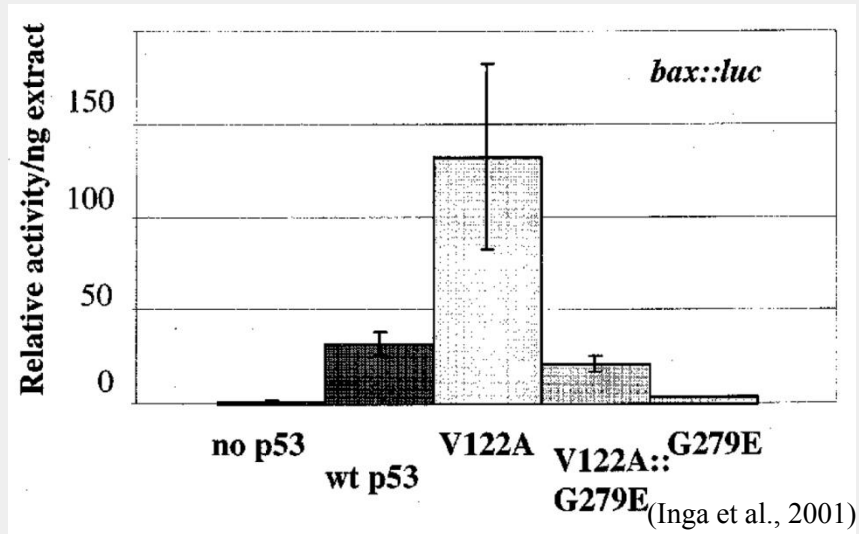


Treatment of $p53^{+/+}$ HCT116 cells with cycloheximide alone resulted in a lower level of p53, which was accompanied by a higher activity of G6PD.

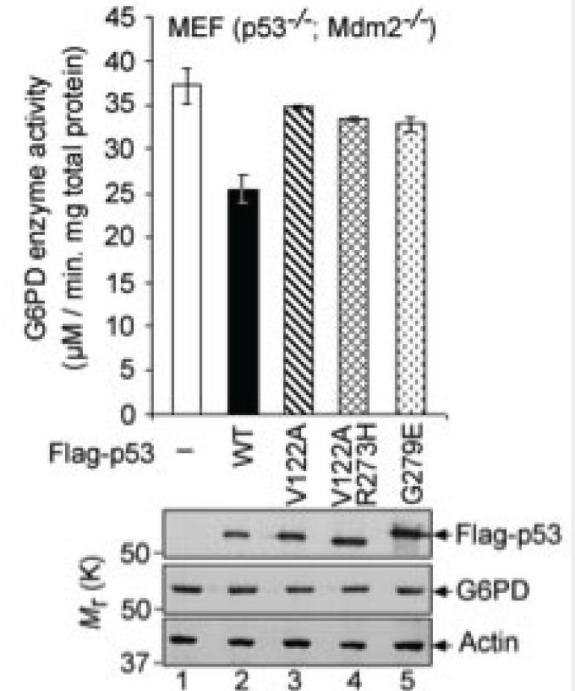
Simultaneous treatment with cycloheximide and doxorubicin led to a stabilization of p53 above the basal level in unstressed cells, and a concurrent drop of G6PD activity below its basal level.

As controls, none of these treatments altered G6PD activity in $p53^{-/-}$ HCT116 cells.

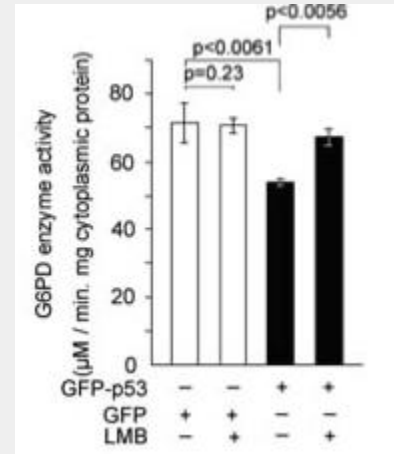
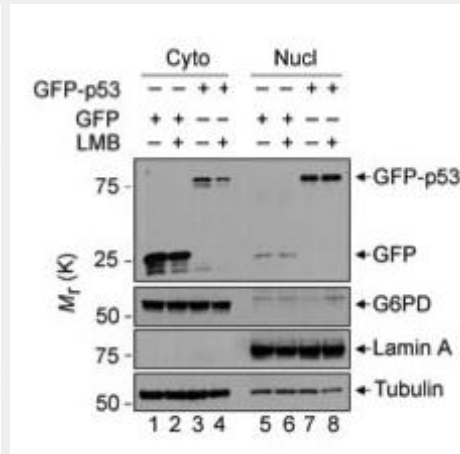
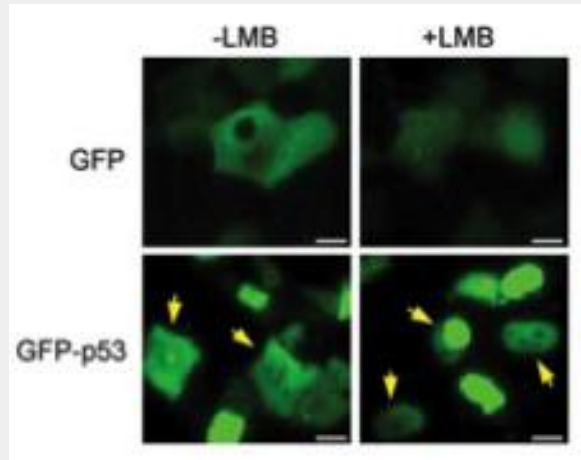
In addition, the p53 mutant V122A, which has a transactivation activity comparable to or even higher than wild-type p53 dependent on the target gene (Inga et al., 2001), **failed to inhibit G6PD**.



b



Moreover, we treated cells with the **nuclear export inhibitor leptomycin B** to **prevent cytoplasmic accumulation of p53**. Leptomycin B reversed p53-mediated inhibition of G6PD.



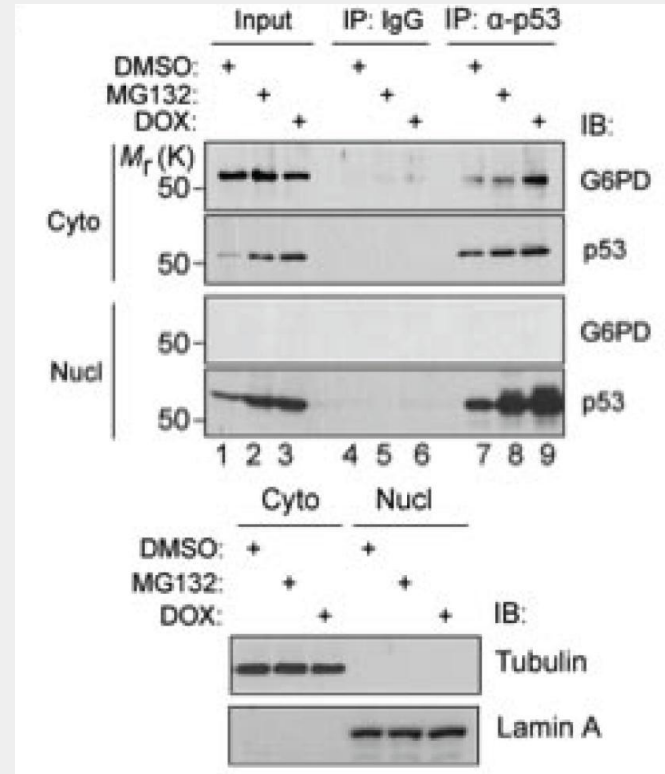
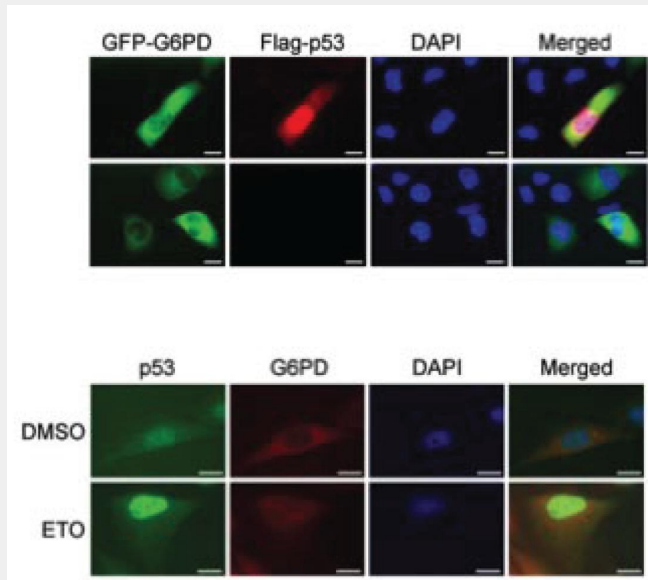
Together, these results show that inhibition of G6PD by p53 is independent of transcription or translation and is **a cytoplasmic, not nuclear, function of p53.**

We next investigated whether p53 interacts with G6PD.

Flag-tagged p53 specifically associated with enhanced green fluorescent protein (eGFP) G6PD *in vivo*. Similarly, endogenous p53 interacted with endogenous G6PD. This interaction was enhanced when cells were treated with the proteasome inhibitor MG132 doxorubicin, both of which stabilized p53.



G6PD is a cytoplasmic protein, whereas p53 is present in both the cytoplasm and the nucleus, and consistently, the p53 - G6PD interaction occurred in the cytoplasm.



谢 谢 聆 听

