Enzymatic Assessment of Cells With Distinct TP53 mutations

Nutritional Sciences

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Abstract

The p53 transcription factor, encoded by the human gene TP53, is the most commonly mutated gene in human cancers. Once the TP53 gene is mutated, p53 proteins not only lose their tumor suppression abilities but can also contribute to tumor growth. One-way mutant p53 can do this is by increasing iron uptake and availability by altering the regulation of iron-sulfur (Fe-S) cluster containing proteins. Understanding such alterations are important because Fe-S cluster proteins play a role in both energy metabolism and DNA repair enzymes, which can suppress the progression and expansion of cancerous tumors. This study's main focus was to examine how TP53 mutation status influences Fe-S cluster protein regulation, and subsequently iron homeostasis in tumor cells. First, we assessed the activity of the Fe-S containing enzymes cytosolic and mitochondrial aconitase in cell lines expressing a variety of TP53 mutations. Specifically, we will use cell lines that express the six most commonly observed TP53 mutations, which represent nearly 25% of all TP53 mutations in human cancers. We found that while aconitase activity decreased as expected following the removal of iron in cells expressing wild-type (WT) TP53, the influence of iron chelation on aconitase activity in cells expressing mutant TP53 varied depending on mutation type. This data strongly suggests that TP53 mutations significantly influence how the activity of Fe-S cluster enzymes is regulated. Thus, our ongoing studies are focused on investigating how TP53 mutation status influences the regulation of genes involved with Fe-S cluster biogenesis and consequently, Fe-S cluster containing protein activity.

Working Hypothesis

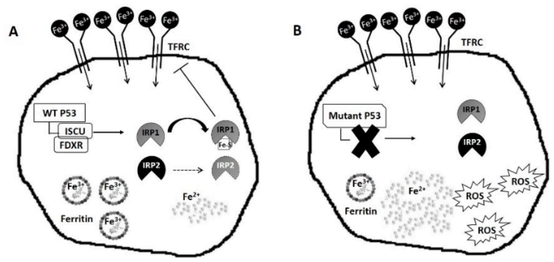

Figure 1. Working model of mutant TP53 dependent alterations in cellular iron homeostasis. (**A**) In response to increased iron availability, wild-type TP53 induces ISCU and FDXR expression, and promotes Fe-S cluster assembly and the reduction of iron regulatory protein (IRP) mRNA binding activity. Decreased IRP mRNA binding activity promotes sequestration of excess iron into ferritin and decreases transferrin receptor (TFRC)-mediated iron uptake. (**B**) In cells expressing mutant TP53, IRP RNA binding activity is unaffected by excess iron, and the labile iron pool, and thus ROS production is significantly increased.

Experimental Approach

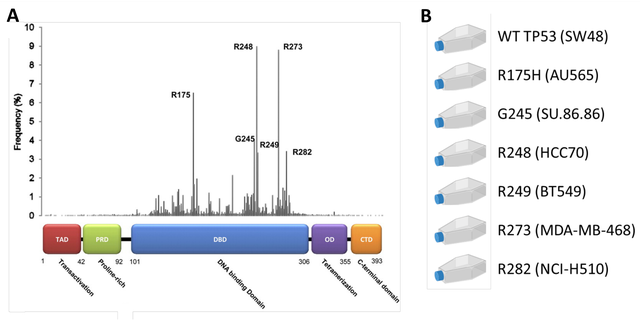

Figure 2. Panel of cell lines representing the six TP53 "hotspot" mutation sites. (**A**) Frequency of TP53 mutation types for all human cancers. (**B**) For this work we obtained a panel of human cancer cell lines that endogenously express either WT TP53, or one of the six most commonly observed TP53 mutation types in human cancers and cultured under standard conditions. Part A of this figure was adapted from: William A. Freed-Pastor, and Carol Prives Genes Dev. 2012;26:1268-1286

Experimental Approach

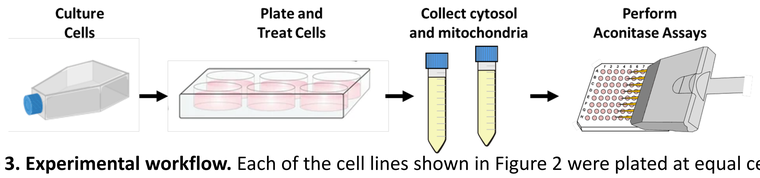

Figure 3. Experimental workflow. Each of the cell lines shown in Figure 2 were plated at equal cell densities and left untreated (control) or were treated with 50 µM or 100 µM of the iron chelator, desferrioxamine (DFO), or 40 µM of the heme iron supplement, hemin, for 24 hours. Cytosolic and mitochondrial subcellular fractionation was then performed by differential centrifugation, and cytosolic and mitochondrial aconitase activities were measured using an Aconitase Activity Assay kit (MilliporeSigma). This assay determines aconitase activity by using a coupled reaction in which citrate is converted to isocitrate by aconitase.

Results

24-hour treatment with DFO or hemin induces iron deficiency and iron overload, respectively

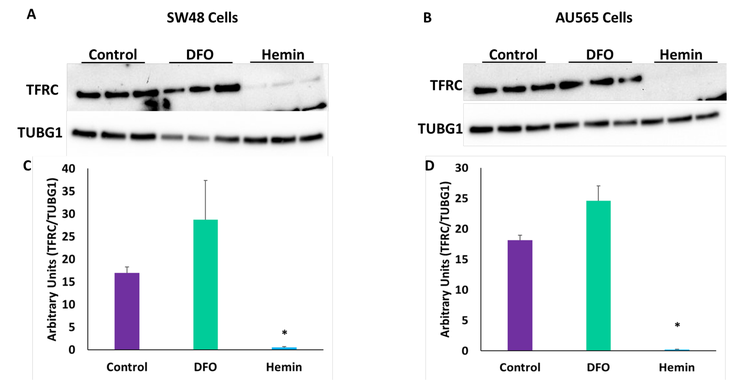

Figure 4. The influence of iron chelation and supplemental iron on TFRC expression in WT and mutant TP53 expressing cells. TFRC expression was assessed by western blot in (**A**) WT TP53 expressing SW48 cells and (**B**) R175H mutant TP53 expressing AU565 cells following treatment with 50 µM DFO or 40 µM hemin for 24 hours. (**C** and **D**). Relative expression levels were quantitated following normalization to anti-gamma tubulin (TUBG1) as the loading control *Denotes statistical difference from respective controls, $p < 0.05$.

The enzymatic activity of Fe-S containing proteins is differentially impacted by iron availability in WT and mutant TP53 expressing cells

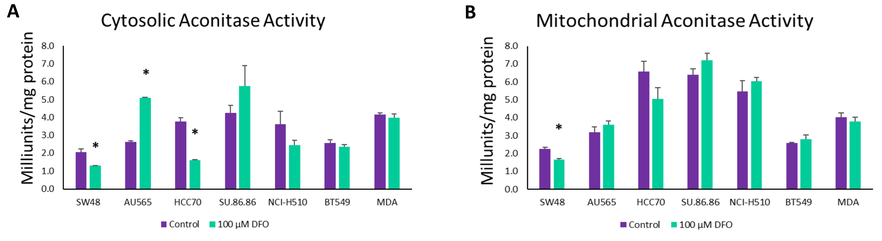

Figure 5. The influence of iron chelation on Fe-S cluster containing enzyme activity in WT and mutant TP53 expressing cells. Cells were treated with 100 μM DFO and untreated (control) for 24 hours. As expected, (**A**) cytosolic aconitase was significantly decreased in cells expressing WT TP53, and the cell line expressing the R248 TP53 mutation type. However, cytosolic aconitase activity in the R175H mutant TP53 expressing cell line actually increased in response to iron chelation. Similarly, (**B**) mitochondrial aconitase activity was reduced in cells expressing WT TP53 but was unaffected in any of the mutant TP53 expressing cell lines examined. *Denotes statistical difference from respective controls, $p < 0.05$.

Conclusions

- Both WT and mutant TP53 expressing cells respond to iron chelation and iron supplementation by increasing or decreasing the expression of the iron uptake protein TFRC.
- In response to reduced iron availability, cells expressing WT TP53 respond by reducing the enzymatic activity of Fe-S containing proteins.
- Most mutant TP53 expressing cell types examined did not appropriately repress the enzymatic activity of Fe-S containing proteins in response to low iron availability.
- Incongruous use of iron in response to alterations in iron availability may represent an exploitable weakness for targeting mutant TP53 expressing cancers.

Future Directions

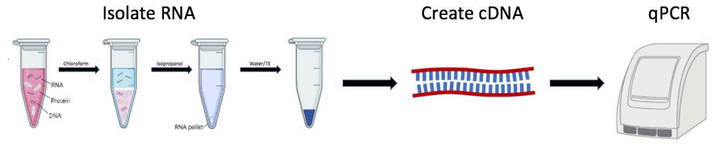

Figure 6. Workflow for next steps of the project. All cell lines will be treated as previously described. RNA will then be isolated and used to synthesize cDNA for qPCR analysis to examine the expression of genes controlling Fe-S cluster biogenesis.

Such studies are important because a mechanistic understanding as to how homeostatic control of iron metabolism is maintained in cancer cells expressing distinct TP53 mutations could lead to improved iron-targeted chemotherapy strategies

Acknowledgements

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