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AUTHOR'S VIEW

Inhibiting system x_C^- and glutathione biosynthesis – a potential Achilles' heel in mutant-p53 cancers

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ABSTRACT

Effective therapeutic strategies to target mutant tumor protein p53 (TP53, best known as p53) cancers remain an unmet medical need. We found that mutant p53 impairs the function of nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, commonly known as NRF2), suppresses solute carrier family 7 member 11 (*SLC7A11)* expression, and diminishes cellular glutamate/cystine exchange. This decreases glutathione biosynthesis, resulting in redox imbalance. Mutant p53 tumors are thus inherently susceptible to further perturbations of the *SLC7A11*/glutathione axis.

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The tumor suppressor protein p53 (TP53, best known as p53) is inactivated in most if not all cancers, commonly via mutation of its coding gene. This confers a selective growth advantage and resistance to conventional anti-cancer therapies. Most *TP53* mutations are missense and typically occur in the central DNA binding domain, which disrupts sequence specific binding to DNA and results in loss of wild-type p53 function. Mutant p53 protein can also accumulate in cells and have gain-of-function activities that contribute to tumourigenesis.[1] Given its central importance, restoration of wild-type p53 function in tumors should trigger growth inhibition and cell death. This is the rationale for the development of therapeutic small molecules such as APR-246 (also known as PRIMA-1^{met}), a drug that is currently showing promise in early-phase clinical trials.[2] Methylene quinuclidinone (MQ), the active derivative of APR-246, covalently binds thiol groups on cysteine residues in the core domain of mutant p53 protein, driving a conformational change resulting in restoration of sequence specific DNA binding, wild-type p53 transcriptional activity and tumor suppressor function.[3] These findings have been confirmed by many subsequent studies across a range of different p53 mutants and cancer types.[4,5] However, a few studies have shown similar therapeutic efficacy of APR-246 in cancer cells without mutant p53 protein.[6] Furthermore, we previously found that knockout of mutant p53 abrogates APR-246 induced cell cycle arrest but not apoptosis,[4] suggesting that additional mechanisms are involved in the anti-tumor activity of this drug.

In a recent study[7] we showed that MQ covalently binds to thiol groups on cysteine residues of glutathione (GSH), which resulted in depletion of intracellular GSH, and increased oxidative stress. This phenomenon was independent of mutant p53 reactivation, but was critical to the therapeutic activity of APR-246. The rate limiting substrate for GSH biosynthesis is intracellular cysteine, the majority of which comes from reduction of cystine, which is imported into the cell by the glutamate/cystine exchanger, system x_C^-. In keeping with these findings, ectopic expression of solute carrier family 7 member 11 (*SLC7A11*), the key component of system x_C^-, increased GSH biosynthesis and induced resistance to APR-246 in cancer cells with *TP53* mutations. Conversely, knockdown of *SLC7A11* increased sensitivity to APR-246 in p53-null cells, demonstrating that *SLC7A11* expression influences APR-246 activity independent of mutant p53 protein. Consistent with this, genome-wide transcriptomic analysis demonstrated that *SLC7A11* expression was the strongest predictor of sensitivity to PRIMA-1, the lead compound for APR-246, thus highlighting *SLC7A11* expression as a potential predictive biomarker for response to APR-246 in addition to *TP53* mutation status. Together, these findings potentially clarify seemingly conflicting reports of APR-246 sensitivity and its relationship to *TP53* mutation and mutant p53 accumulation. That is, inherent defects in GSH biosynthesis, such as low expression of *SLC7A11*, may explain reports of APR-246 induced cell death in the absence of mutant p53 protein or the restoration of wild-type p53 transcriptional activity.

Why then, given the ubiquitousness of GSH in cells and its importance as the major intracellular antioxidant, are cancer cells with mutant p53 protein generally more sensitive to APR-246? The answer lies in the novel inverse relationship between *SLC7A11* expression and mutant p53 accumulation that we

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identified in our esophageal adenocarcinoma models, and confirmed in other tumor types using The Cancer Genome Atlas database.[7] Utilizing genetic approaches we established that this inverse relationship is mediated by an interaction between mutant p53 protein and nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, commonly known as NRF2), which impairs NRF2-mediated transcription of target genes involved in redox regulation, including *SLC7A11*. Thus, accumulation of mutant p53 protein supresses *SLC7A11* expression leading to increased basal oxidative stress and reduced cellular capacity to detoxify reactive oxygen species (Fig. 1a). As a consequence, mutant p53 effectively sensitizes cancer cells to oxidative stress resulting from further depletion of GSH by APR-246. Similarly, genetic or pharmacological inhibition of *SLC7A11* creates a synthetic lethal interaction with mutant p53 accumulation,[7] raising the potential for a new therapeutic paradigm to target cancers with accumulated mutant p53 that is analogous to the use of PARP inhibitors in BRCA deficient cancers. While this is a potential weakness that might be predicted to be selected against during tumor evolution, increased basal oxidative stress induced by mutant p53 may have pro-oncogenic effects, including increased oxidative DNA damage leading to genomic instability.[8] Therefore, together with the loss of wild-type p53 tumor suppressor activity, we propose that this function of mutant p53 may instead provide a selective advantage during tumourigenesis (Fig. 1a).

The interaction between NRF2 and mutant p53 has been confirmed by others[9] where, remarkably, it promotes NRF2-mediated expression of proteasome machinery, leading to degradation of multiple tumor suppressors and contributing to resistance to proteasome inhibitors. Significantly, Del Sal and colleagues show that APR-246 disrupts the interaction between mutant p53 and NRF2, thereby down-regulating proteasome gene expression and restoring sensitivity to proteasome inhibitors.[9] As would be predicted based on this finding, expression of *SLC7A11* and other anti-oxidant gene targets of NRF2 are upregulated in cancer cells with mutant p53 protein following treatment with APR-246,[10] which has the potential to negate the therapeutic activity of APR-246 mediated through GSH. This provides mechanistic rationale for combining APR-246 with inhibitors of the system x_C^-/GSH axis (Fig. 1b). Indeed, antagonising SLC7A11 in combination with APR-246 selectively and synergistically induces cell death in tumors with mutant p53 accumulation.[7]

Overall, our study has uncovered a potential Achilles' heel in cancers with accumulated mutant p53 and a novel paradigm

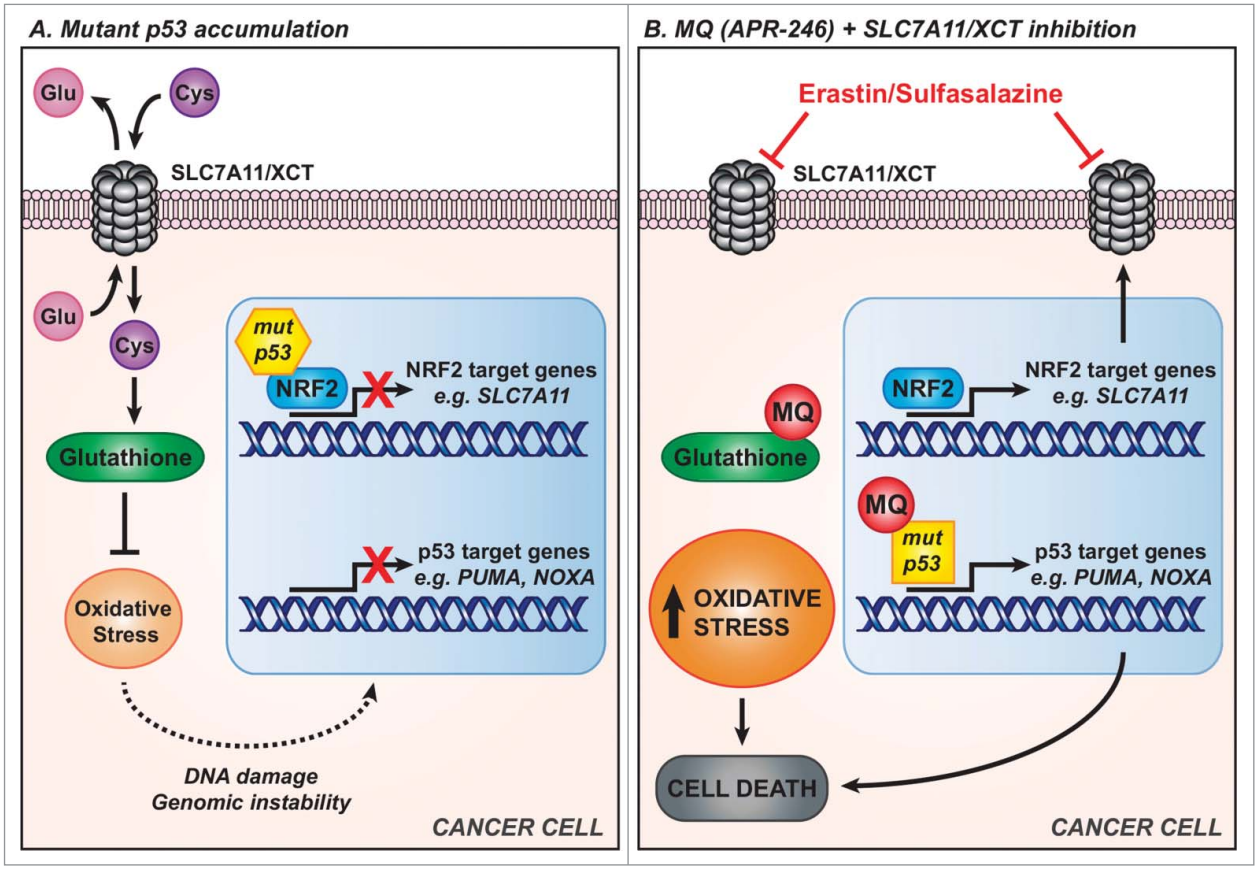

Figure 1. Accumulation of mutant tumor protein p53 (TP53) raises basal oxidative stress and induces susceptibility to glutathione depletion (a) Accumulation of mutant TP53 (shown as mutp53) in cancer cells impairs nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, best known as NRF2) function and reduces the expression of NRF2 target genes, including solute carrier family 7 member 11 (*SLC7A11*, also known as xCT), the cystine (Cys)/ glutamate (Glu) anti-porter. This results in reduced glutathione synthesis and higher basal oxidative stress compared with normal cells. In the absence of wild-type p53 tumor suppressor function, increased oxidative stress likely contributes to tumourigenesis via oxidative DNA damage and genomic instability. (b) As a consequence, cancer cells with accumulation of mutant p53 protein are sensitive to the glutathione depleting effects of methylene quinuclidinone (MQ, the active derivative of APR-246) or inhibition of SLC7A11. Binding of MQ also restores wild-type p53 transcriptional activity to mutant p53 and disrupts the interaction between mutant p53 and NRF2. This latter effect results in upregulation of *SLC7A11* in response to oxidative stress, providing the mechanistic rationale for combining APR-246 with SLC7A11 inhibitors.

for mutant p53 directed anti-cancer therapies. Our novel insights into the mechanism of action of APR-246 and unification of our understanding of what drives APR-246 sensitivity suggest clear criteria for patient selection and rational drug combinations, with the capacity to be immediately translated into clinical trials.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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