# Oncoprotective Role of Chelation Therapy in Hypoxic Solid Tumors: A Theoretical Model

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***Abstract*—Solid tumors show areas of differential blood supply due to decreasing vascularisation from periphery towards the core. As a result, a gradient of oxygen is formed such that internal areas with low oxygen tension lead to altered physiochemical conditions. This state of low oxygen- called hypoxia- is gaining interest due to a large scale shift in metabolism of the tumor cells. Reactive Oxygen Species (ROS) thus produced cause extensive DNA damage in hypoxic core regions, exacerbating the aggressiveness of cancer by accumulation of mutations. We show how the ubiquitous cellular enzyme Catalase may prevent ROS mediated damage through chelation of metal ions. Metal ions are known to increase oxidative stress in a cell and recent reports implicated involvement of metal ions in controlling p53 function. We propose that chelation therapy in solid tumors can delay the accumulation of mutations by catalase mediated decrease in ROS levels and by rescuing the p53 activity in a cell.**

***Index Terms*—Catalase, chelation, hypoxia, oxidative stress.**

## I. Introduction

Cancer is a multigenic process which involves the accumulation of several mutations which offer a competitive advantage to the cancerous cell over normal cells. The accumulation of mutations in the DNA relate to the pathogenesis of the disease. However, a number of cellular mechanisms- called cell cycle checkpoints- ensure that an abnormal cell does not proceed towards cell division either by forcing these cells into cell-cycle arrest or apoptosis[1]. Multiple mutations are thus required to transform a normal cell into a cancer cell that increase the cancer's clinical aggressiveness- that is conversion from the relatively innocuous benign tumors to debilitating metastatic cancers. Also, a loss of heterozygocity (LOH) [2] is seen in cancers which impair both alleles of genes which otherwise show normal phenotype in heterozygous state.

Solid tumors are cancerous growths in the form of sarcomas, carcinomas and lymphomas that contain an abnormal mass of tissue devoid of liquid area[3]. Rapid proliferation of cells that form the tumor cause internal areas to be poorly vascularized [4]. The low blood supply established to internal areas of the tumor causes a shortage of various nutrients including oxygen. The decrease in oxygen supply from about 2.5-9 percent oxygen tension to less than 1 percent is called hypoxia [5]. As oxygen is critical for cellular energetics, hypoxia is reported to alter metabolic state of a cell and hence a marked level of oxidative stress is seen due to abnormal metabolism during hypoxia [6]. The molecular species that generate oxidative stress are called Reactive Oxygen Species (ROS) and include superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$) and nitric oxide ($NO^{\cdot}$) in addition to hydrogen peroxide ($H_2O_2$) and peroxynitrite ($ONOO^-$) [7]. These are highly unstable species that react with lipids, proteins, RNA and DNA. While other biomolecules undergo rapid turnover, DNA damage caused by ROS increases chances of cancerous transformations as DNA can only be repaired with limited efficiency. Thus, ROS generation during hypoxic conditions in solid tumors predispose the cells towards accumulation of more aggressive mutations.

Cells possess several enzymes that utilize the ROS species as substrates and form products that are harmless to the cell. The various enzymes include catalase, superoxide dismutase and glutathione. Our study involves the use of the enzyme catalase. Catalase catalyses the breakdown of hydrogen peroxide to water and molecular oxygen. This enzyme has one of the highest turnover number in that it catalyses the breakdown of millions of substrate molecules to products per second [8]. This activity is critical to reduce the oxidative load on a cell and prevents ROS mediated DNA damage. Catalase has an active heme-containing porphyrin center that catalyses $H_2O_2$ breakdown. It is sensitive to concentration of metal ions: some metal ions reduce catalytic activity of catalase.

Metal ions exhibit noxious effects by increasing the cellular ROS levels. These are an outcome of a phenomenon called redox cycling [9] which involves transfer of free radicals from a series of compounds to a final recipient like an anti-oxidant molecule. However, metal ions interfere within the cycle and cause free radical transfer to form peroxides. These metals include iron (Fe), copper (Cu), chromium (Cr), cobalt (Co) etc. The ROS damages DNA directly or as in several cases, the formation of products such as malondialdehyde (MDA), 4-hydroxynonenal (HNE) form DNA adducts. Interestingly, the role of zinc (Zn) is reported to be critical in DNA damage; low levels of zinc impair DNA repair mechanisms. Recent findings indicate possible control of p53 by metal ions by interfering with p53 function [10]. In this regard, copper has been shown to bind to p53 protein thereby altering its conformation and DNA-binding activity implying a possible control of p53 by this ion [11].

The p53 tumor suppressor prevents malignant transformations by activating genes that cause cell cycle arrest or apoptosis [12]. During unstressed conditions, a cell constantly degrades p53 by continuous degradation of this constitutively expressed protein through the MDM2 ubiquitination pathway. Since p53 activation has sensitive roles in a cell, it is under strict control itself. Under stress, p53

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levels get upregulated by inhibition of its degradation [13]. Several mechanisms lead to p53 upregulation. As mentioned, metal ions interfere with p53 function leading to compromised p53 activity. Reduction in p53 activity affects critical decisions such as those between cell cycle arrest or cell death; an incorrect decision to arrest an irreversibly damaged cell may increase chances of oncogenic transformations.

As a possible prevention of the ROS mediated damage to DNA in hypoxic cores of a tumor [14], we show *in vitro* how catalase activity is affected by various concentrations of metal ions and how in the presence of a chelating agent EDTA ( Ethylene Diamine Tetra Acetic acid), this activity can be enhanced. The chelation of metal ions in hypoxic tumor areas shall reduce ROS levels, increase catalase activity and may prevent metal ion mediated reduction of p53 activity delaying the formation of aggressive cancers. This may increase the clinically critical remission periods.

## II. Procedure for Catalase Isolation and Measurement of Catalase Activity

### A. Preparation of the Crude Extract

A fresh potato tuber was peeled and cut into small cubes. 50 g of potato cubes were then weighed on a Finemech Benchscale GAB weighing balance. The potato cubes along with 50 ml of cold distilled water and 50 ml crushed ice was homogenized for 30 seconds at high speed. The enzyme preparation was now carried on an ice bath to retain enzyme activity.

### B. Preparation of Catalase Enzyme Solution

The potato extract was filtered and the filtered solution was poured into a 200 ml graduated cylinder. 40 ml of chilled Acetone (supplied by Fisher Scientific) is added slowly to the solution. This is again filtered through Whatman No. 12 filter paper and kept overnight at $4^0$C. To the same solution, 20 ml of chilled acetone is added again, and a precipitate can be seen. This precipitate contains catalase. The liquid is decanted and distilled water is added to make final volume to about 50 ml. The resultant was a 100 % enzyme solution. The catalase preparation should be kept on ice.

### C. Preparation of Hydrogen Peroxide Solution with Metal Ions and EDTA

10% $H_2O_2$ solution was used for the study. 5ml of 10% $H_2O_2$ is added to each bottle and the metal salts are added along with EDTA in a concentration as indicated in the TABLE I. Proper time is given for the salts and EDTA to be dissolved.

TABLE I: The Various Metal Ions The Desired Final Concentrations.

| S.No | Salt | Mol. mass | Ion produced | Conc. M(1) | Conc. M(2) |
|---|---|---|---|---|---|
| 1 | $Al(NO)_3$ | 213g | $Al^{3+}$ | 0.01 | 0.01 |
| 2 | $MnSO_4$ | 150g | $Mn^{2+}$ | 0.01 | 0.01 |
| 3 | $MgSO_4$ | 120g | $Mg^{2+}$ | 0.01 | 0.01 |
| 4 | $CaCl_2$ | 111g | $Ca^{2+}$ | 0.01 | 0.01 |
| 5 | EDTA | 372g | - | 0.01 | 0.01 |

### D. Measuring the Enzyme Activity using the Filter Disc Method

The filter disc method was used to measure enzyme activity. A filter paper disc was soaked in the catalase enzyme solution and immersed in the solution. The time it took to reappear onto the surface (in seconds) gave a measure of the enzyme activity. It should be noted that normally a disc would take about 20 seconds to rise in a beaker at room temperature (approx $20^0$ C) in a 10 % $H_2O_2$ solution.

## III. Results

The results are presented in the form of TABLE I and TABLE II. As can be seen from the table, a concentration of .01M EDTA slightly more effectively enhances catalase activity that at .001M EDTA. This may be because at .001 M EDTA, many metal ions are still free to interact with catalase and reduce activity. An abnormal trend is seen for Magnesium ions, which when withdrawn from the solution by EDTA, shows a negative effect on catalase activity, implying that these play an essential role in increasing the activity of catalase.

TABLE II: This Table Shows the Activity of CATALASE in The Presence of Listed Metal ions in The Presence of .01M EDTA as Compared to Its Control (Control Has No Added EDTA). the Table Also Shows The Percent Decrease in CATALASE Activity.

| S.No | Ion (.01M) | Conc of EDTA M(1) | Activity (in Sec) | Control (in Sec) | Percent Change in Activity |
|---|---|---|---|---|---|
| 1 | $Al^{3+}$ | 0.01 | 35 | 42 | 16 |
| 2 | $Mn^{2+}$ | 0.01 | 70 | 201 | 65 |
| 3 | $Mg^{2+}$ | 0.01 | 54 | 46 | 26 |
| 4 | $Ca^{2+}$ | 0.01 | 40 | 60 | 33 |

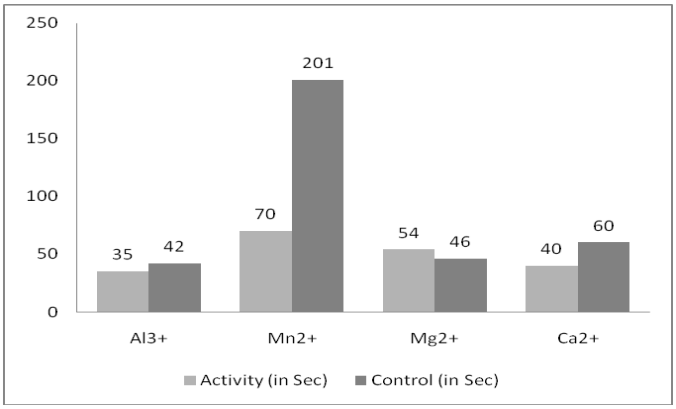


Fig. 1. The Graph shows the change in activity of catalase with various metal ions and .01M EDTA compared to control.

TABLE III: this Table Shows the activity of CATALASE in the Presence of Listed Metal Ions In The Presence of .001M EDTA as Compared to ITS CONTROL (CONTROL HAS NO Added EDTA). the Table Also Shows The Percent Decrease in CATALASE Activity.

| S.No | Ion (.01 M) | Conc of EDTA M(2) | Activity (in Sec) | Control (in Sec) | Percent Change in Activity |
|---|---|---|---|---|---|
| 1 | $Al^{3+}$ | 0.001 | 32 | 41 | 22 |
| 2 | $Mn^{2+}$ | 0.001 | 88 | 205 | 57 |
| 3 | $Mg^{2+}$ | 0.001 | 46 | 40 | 15 |
| 4 | $Ca^{2+}$ | 0.001 | 38 | 60 | 36 |

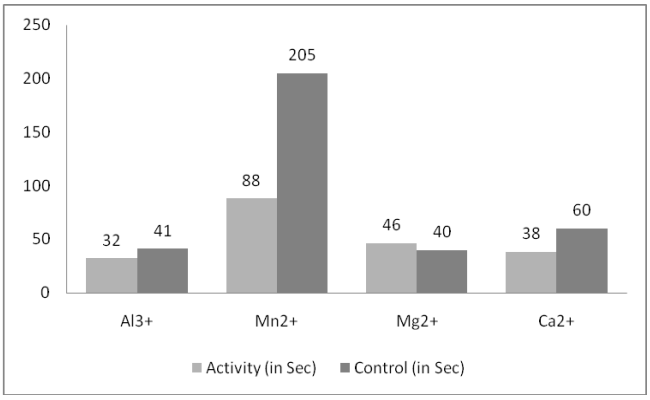


Fig. 2. The Graph shows the change in activity of catalase with various metal ions and .001M EDTA compared to control.

## IV. Conclusion

We show that many metal ions including aluminium, manganese and calcium decrease the activity of the enzyme catalase, while the ions of magnesium showed a slight increase in catalase activity. In light of previous research that has shown the critical link between the presence of various metal ions and a resulting increase in the ROS generation, we report that a catalase based chelation therapy by various ligands is successful in breakdown of peroxide. We further propose that this therapy may be successful in treatment of hypoxic tumor areas which show increased oxidative stress possibly advancing cancer stages. Such a chelation therapy to increase catalase activity will reduce ROS load on a cell. The reduction of metal ion concentrations from cells will restore p53 function, which is known to be abolished in the presence of ions such as copper, by rescuing the native conformation and DNA binding ability of p53.

We show concentration dependent drop in catalase activity with EDTA. Absolute activity for catalase is minimum for manganese and maximum for aluminium. Highest percent inhibition is shown by manganese while aluminium and magnesium seem relatively less affected by the chelating effect of EDTA. Calcium, which is abundant in various compartments of a cell and is a critical intracellular signalling molecule, is a promising candidate for chelation therapy. Manganese shows best restoration of catalase activity, but since its physiological concentration is very less, it may not be a suitable therapeutic target for hypoxic cancers.

## V. Future Prospects

The chelation therapy proposed here may be combined with chemotherapy and tested on animal models to evaluate tumor regression and possibly for clinical trials.

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