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**IRON OVERLOAD AS AN ALTERNATIVE MECHANISM OF INDUCING TOXICITY  
IN CANCER CELLS**

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**ABSTRACT**

The world is well informed of the efficacy of iron (Fe) chelators in chelating Fe in case of excessive Fe accumulation in the body. In fact, this efficacy of Fe chelators is widely used to inhibit several types of cancer cell progression such as human neuroblastoma and human breast cancer cells [1, 2]. In this article, we are driven to lure the world's attention on an interesting, promising approach to induce toxicity in cancer cells, through the induction of Fe overload as an alternative mechanism of cancer therapeutics, instead of focusing on a well-recognised Fe deprivation mechanism to inhibit cancer cell proliferation.

**Keywords: Iron, Iron Overload, Nitric Oxide, Cancer Cells**

**INTRODUCTION**

Virtually 5% of the earth's crust is composed of Fe [3]. This puts Fe as the fourth most abundant element in the world and the second most abundant metal in the earth [4, 5]. In the human body, Fe is the most abundant transition metal with 3 to 4 g per individual (~50 mg/kg) [6, 7]. The majority of this Fe is

distributed in the haemoglobin of red blood cells and developing erythroid cells, followed by a significant amount of up to 600 mg scattered in macrophages, 300 mg in the myoglobin of muscles, and the excess of about 1 g is stored in the liver, which is the major depot of Fe [8].

The great abundance of Fe is coupled with the fact that all living organisms have an absolute requirement for Fe [9]. This element of Fe is essential for mammals as enzymes require Fe as a cofactor for metabolic processes. It has been suggested that the first step in the origin of life is catalysed by reactions that involved Fe. This explains the essential nature of Fe in cell growth. Fe is an absolute requirement for cells to grow, as Fe-containing proteins catalyse key reactions that are involved in fundamental biochemical activities, such as oxygen transport, energy metabolism, respiration and DNA synthesis [10]. Moreover, without Fe, cells are unable to proceed from the G<sub>1</sub> to the S phase of the cell cycle. This is the crucial checkpoint which is regulated by the Fe-containing enzyme ribonucleotide reductase [11]. This enzyme is involved in the synthesis of DNA, where Fe serves as a cofactor to convert ribonucleotides to deoxyribonucleotides [12]. Therefore, if Fe is deficient in cells, both DNA synthesis and cell proliferation are inhibited, which results in a retardation of cell growth.

Fe commonly exists in two redox states; ferrous (Fe<sup>2+</sup>) and ferric (Fe<sup>3+</sup>), both of which are able to donate and accept electrons, which allows Fe to participate in electron transfer reactions [13]. This flexible coordination chemistry and redox reactivity have allowed

Fe to associate with proteins and regulate the functionality of biological systems. One of the major cellular systems (e.g. mitochondrial respiratory chain) is dependent on Fe for its functionality. With a lack of Fe, this system will be affected. Besides, the susceptibility of cells to oxidative damage will also increase under Fe deprivation conditions. Therefore, Fe regulation is extremely important to maintain Fe homeostasis as Fe overload and Fe deprivation are cytotoxic. Fe overload can cause oxidative damage via excessive production of reactive oxygen species (ROS). On the other hand, Fe deprivation can inhibit cell growth and lead to apoptosis. Excessive exposure of cellular proteins to ROS will disrupt cellular Fe regulation by inducing Fe trafficking in cells. Therefore, potentially both Fe overload and Fe deprivation are beneficial for cancer therapy if they could be selectively induced within a tumour.

### **Fe Overload**

The augmentation of Fe uptake may cause Fe overload in the body. This occurs when the total amount of Fe in the body exceeds 5 g. Studies have shown that Fe overload is harmful to humans. Those who suffer from hereditary hyperferritinaemia cataract syndrome, who have high levels of Fe stored in ferritin, have been discovered to have high oxidative damage in the eyes [14]. Non-

anaemic adults who have been supplemented with Fe have been shown to have Fe overload and overproduction of pro-oxidants in their bodies [15].

The accumulation of free Fe initiates lipid peroxidation in cellular organelles [16]. For example, lipid peroxidation in the mitochondria modifies the permeability state of the membrane and alters solutes and ion transportation, leading to swelling and lysis of the mitochondria [17]. This leads to the damage of microsomes that reduces cytochrome activity [18]. Mitochondrial oxidative metabolism in the Fe overload condition indicates a decrease in mitochondrial respiratory control ratio, suggesting Fe-induced mitochondrial lipid peroxidation has occurred.

An excessive amount of Fe also initiates lysosomal membrane lipid peroxidation. It increases the lysosomal fragility that interferes with normal fusion of lysosomes with the canalicular membranes [19]. Such alterations inhibit the lysosomal membrane proton pump and increase the intralysosomal pH levels, leading to lysosomal disruption and ultimately in cell death. The participation of Fe in lipid peroxidation indicates that Fe needs to always be chaperoned in order to limit oxidative damage.

### **Targeting Fe Overload For Fe-Induced Cytotoxicity. The Intervention of Nitric Oxide (NO<sup>•</sup>)**

There is a close link exists between NO<sup>•</sup> and Fe homeostasis. NO<sup>•</sup> has been reported to disrupt Fe regulation in normal and cancer cells including fibroblast, macrophages, lung and leukaemia cells [20, 21, 22, 23]. In particular, the switch of iron regulatory protein (IRP) functions between the iron responsive element (IRE) binding activity and the aconitase activity can be operated by NO<sup>•</sup> [24, 25]. NO<sup>•</sup> has been shown to increase the stability of transferrin receptor (TfR) mRNA against targeted degradation, which in return inactivates aconitase activity [26, 27].

The stabilisation of TfR mRNA causes an increase in TfR protein expression while repressing ferritin mRNA translation, both of which are proteins involved in regulating Fe levels in the body [28]. TfR is responsible for mediating the uptake of Fe into the cells whereas ferritin is responsible for the intracellular Fe storage. The expressions of both proteins are important for maintaining a balanced Fe homeostasis [29]. (See **Figure 1** for a brief picture of the involvement of TfR and ferritin in the mechanism of Fe transportation).

In response to increased cellular Fe levels, Fe will accumulate inside IRP and assemble a

cubane [4Fe-4S] cluster (**Figure 2**). The cluster assembly renders the IRP to be a cytosolic aconitase, which prevents the binding on IRE binding sites of TfR mRNA [30, 31, 32]. As a result, this will down-regulate the expression of TfR protein due to destabilisation of TfR mRNA. Concomitantly, this increases the ferritin Fe storage in cells as a result of increased ferritin translation. On the other hand, when cells are deprived of Fe, IRP will have a high affinity for IRE binding sites of TfR mRNA. The uptake of Fe into the cells will be increased by up-regulating the transcription of TfR protein [33] (See **Table 1** for the whole event summarisation).

Having said that NO<sup>•</sup> has the capability of operating the switch between the IRE binding activity and the aconitase activity, therefore, we could use NO<sup>•</sup> to selectively tricking cancer cells into continuously taking Fe, and ultimately causing Fe overload in cancer cells. In this process, which mimicking the condition of Fe deprivation, NO<sup>•</sup> exposure will cause a disruption of [4Fe-4S] cluster, and aconitase enzyme will be converted back into IRP form, allowing the cancer cells to increase the uptake of Fe. Hypothetically, this process will lead to the ultimate cell death from the formation of excessive ROS due to the accumulation of free Fe in cancer cells (**Figure 3**).

## CONCLUSION

The hypothetical mechanism of inducing Fe overload in this article is the effect mimicking the consequence of Fe starvation. The molecular mechanism involves the interaction of NO<sup>•</sup> with metal Fe in the centre of aconitase and causes a removal of labile Fe atom in the [4Fe-4S] cluster. This results in the conversion of aconitase enzyme into IRP to increase the transcription of TfR mRNA so that Fe is continuously taken up to meet the cellular demand until it is reaching the limit that will cause oxidative damage from excessive accumulation of free Fe ions. Therefore, this interaction suggests a potential mechanism by which NO<sup>•</sup> mediates cytotoxicity through the induction of Fe overload. Taking into account the significant involvement of NO<sup>•</sup> in biological processes, especially in regulating cellular Fe metabolism, there has been a remarkable increase in research, conducted to generate synthetic compounds that can release NO<sup>•</sup> *in vivo*. Study is underway to elucidate the potential pathway involving NO<sup>•</sup> in increasing intracellular Fe levels.

## ACKNOWLEDGEMENT

The author would like to thank the Universiti Teknologi MARA (UiTM) for funding and Dr. Gregory Giles from the University of

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Otago, New Zealand for the advice and guidance throughout the study.

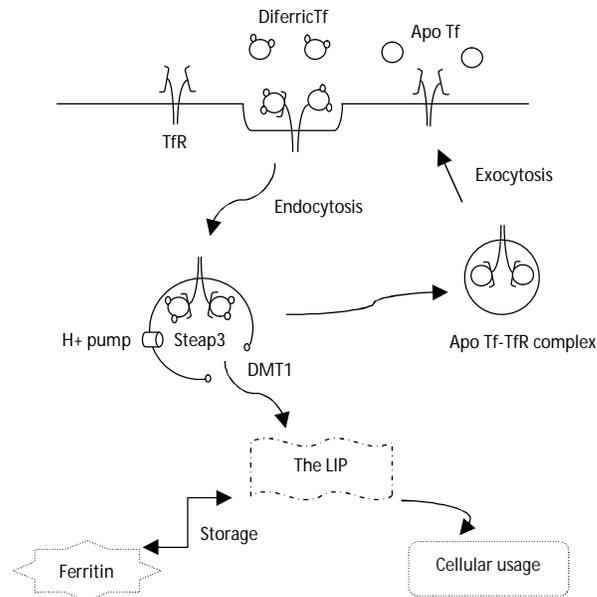
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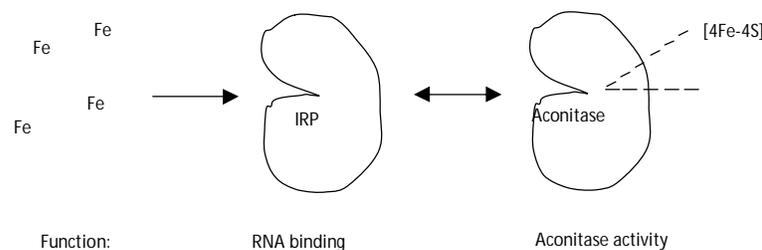
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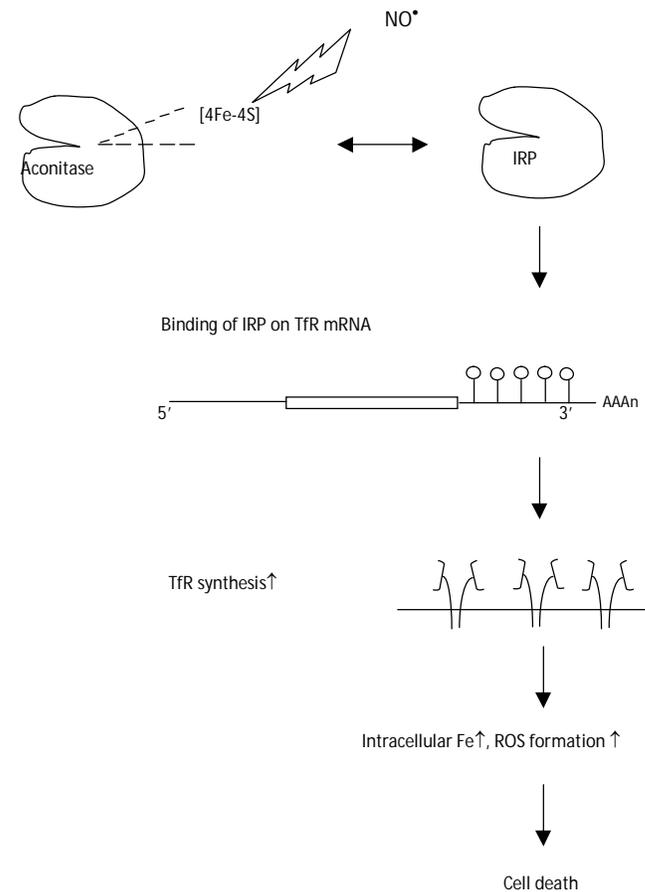
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**Figure 1: Mechanism of Fe transportation.** Primary Fe uptake occurs via Tf-TfR-mediated endocytosis. A proton pump acidifies the endosome, causing Fe to be released from the Tf-TfR complex. Fe is reduced by Steap3 and transported across the endosomal membrane to the cytosol via DMT1. Fe in the LIP is directed to the cellular constituents for metabolic utilisation, or transported into ferritin for storage. The apo-Tf-TfR complex is exported to the cell surface by exocytosis and Tf is released from TfR to bind other Fe ions.



**Figure 2: Schematic representation of Fe regulation by IRP.** In a normal physiological condition, Fe will accumulate inside IRP and assemble an [4Fe-4S] cluster, allowing the conversion of IRP into aconitase enzyme, which will prevent the IRP binding activity on the IRE binding site of TfR mRNA



**Figure 3: Hypothetical mechanism to trick cancer cells to induce Fe overload. Upon NO• exposure, [4Fe-4S] cluster will be disrupted, thus preventing aconitase activity and rendering the enzyme to be converted back into IRP form. This conversion is consequently tricking cancer cells that they have a lack of Fe, a process mimicking Fe deprivation condition, and therefore TfR mRNA will be further transcribed. Synthesising TfR allows Fe to be continuously taken up into the cells, eventually leading to Fe overload and cell death**

**Table 1: Summarisation of Events Occurs During Fe Regulation in the Body**

Event	Low Fe	High Fe
IRP binding activity	↑	↓
Aconitase activity	↓	↑
Ferritin synthesis	↓	↑
TfR synthesis	↑	↓