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Review

Expanding horizons in iron chelation and the treatment of cancer: Role of iron in the regulation of ER stress and the epithelial–mesenchymal transition

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ABSTRACT

Cancer is a major public health issue and, despite recent advances, effective clinical management remains elusive due to intra-tumoural heterogeneity and therapeutic resistance. Iron is a trace element integral to a multitude of metabolic processes, including DNA synthesis and energy transduction. Due to their generally heightened proliferative potential, cancer cells have a greater metabolic demand for iron than normal cells. As such, iron metabolism represents an important "Achilles' heel" for cancer that can be targeted by ligands that bind and sequester intracellular iron. Indeed, novel thiosemicarbazone chelators that act by a "double punch" mechanism to both bind intracellular iron and promote redox cycling reactions demonstrate marked potency and selectivity *in vitro* and *in vivo* against a range of tumours. The general mechanisms by which iron chelators selectively target tumour cells through the sequestration of intracellular iron fall into the following categories: *(1)* inhibition of cellular iron uptake/promotion of iron mobilisation; *(2)* inhibition of ribonucleotide reductase, the rate-limiting, iron-containing enzyme for DNA synthesis; *(3)* induction of cell cycle arrest; *(4)* promotion of localised and cytotoxic reactive oxygen species production by copper and iron complexes of thiosemicarbazones (*e.g.*, Triapine® and Dp44mT); and *(5)* induction of metastasis and tumour suppressors (*e.g.*, NDRG1 and p53, respectively). Emerging evidence indicates that chelators can further undermine the cancer phenotype *via* inhibiting the epithelial–mesenchymal transition that is critical for metastasis and by modulating ER stress. This review explores the "expanding horizons" for iron chelators in selectively targeting cancer cells.

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Abbreviations: ASK1, apoptosis signal-regulating kinase 1; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; Bcl-2, B-cell lymphoma 2; BiP, binding immunoglobulin protein; bZIP, basic leucine zipper; CDK, cyclin-dependent kinase; CDKI, CDK inhibitors; CHOP, C/EBP homologous protein; DFO, desferrioxamine; dNTP, deoxyribonucleotide triphosphate; Dp44mT, di-2-pyridylketone 4,4-dimethyl-3- thiosemicarbazone; eIF2α, eukaryotic initiation factor 2α; EMT, epithelial–mesenchymal transition; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GADD34, growth arrest DNA-damage inducible gene 34; GSK-3, glycogen synthase kinase 3; HIF, hypoxia inducible factor; IRE1, inositol-requiring enzyme 1; JNK, c-Jun *N*-terminal kinase; MEFs, mouse embryonic fibroblasts; MET, mesenchymal–epithelial transition; NDRG1, N-myc down-stream regulated gene 1; NF-κB, nuclear factor of κ light polypeptide gene enhancer in B-cells; p53, tumour suppressor protein 53; p53R2, p53-inducible RR; $p58^{IPK}$, protein 58 inhibitor protein kinase; PERK, protein kinase-like ER kinase; Rb, retinoblastoma protein; RR, ribonucleotide reductase; R1, RR subunit 1; R2, RR subunit 2; ROS, reactive oxygen species; siRNA, small interfering RNA; SMAD, mothers against decapentaplegic homolog; TGF-β, transforming growth factor β; TNF, tumour necrosis factor; TRAF2, TNF-receptor associated factor 2; UPR, unfolded protein response; XBP1, X box binding protein-1

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1. Introduction

Cancer is a debilitating disease that, despite years of research, remains a leading cause of death in developed countries [1]. While "cancer" is an umbrella term covering a broad group of diseases, which typically have different aetiologies, outcomes and mechanisms of propagation, all cancers are characterised by uncontrolled and/or abnormal cellular proliferation. Estimates made in 2008 indicate that 12.7 million new cancer cases were diagnosed and 7.6 million cancer deaths occurred worldwide [1]. The continuing growth and ageing of the world's population will result in an ever-increasing global cancer burden. For example, it has been estimated that the number of new cancer cases will increase to 22.2 million by 2030, thereby becoming the global leading cause of death, regardless of region or socio-economic status [2]. While this predicted rise in cancer burden will largely be due population expansion and ageing, the limitations of our current therapeutic arsenal will only serve to exacerbate this looming problem.

Current cancer treatments consist of surgery, chemotherapy, radiotherapy and immunotherapy, either alone or in combination. The main limitation of these therapeutic modalities is the inability to differentiate between normal, healthy cells and cancerous cells. As a result, severe side effects, such as nausea, vomiting, loss of appetite and alopecia are inherent, particularly with chemotherapeutics [3]. In addition, the DNA damage that some anti-cancer drugs induce can lead to carcinogenicity [4], potentially turning healthy cells malignant or increasing the malignancy of already cancerous cells.

Recent research into iron chelation therapy has demonstrated promising novel anti-cancer mechanisms [5]. Iron plays a major role in many crucial biological systems, in particular DNA synthesis, cell growth and proliferation, making it an effective target for anti-cancer chemotherapeutics [6,7]. Recent advances in iron chelation have demonstrated significant metastasis suppression mechanisms, many of which putatively operate through an up-regulation of the iron-regulated metastasis suppressor, N-myc down-stream regulated gene 1 (NDRG1) [8]. As will be discussed further in this review, the molecular targeting of this regulatory protein has greatly extended our understanding of the effect of iron chelators into numerous oncogenic pathways, including NDRG1-dependent modulation of proliferative signals, endoplasmic reticulum (ER) stress pathways and the inhibition of key initiating steps of metastasis [9–11]. This is of particular interest, especially since it is the metastases, not the primary tumour, that accounts for 90% of cancer deaths [12].

2. Iron and cellular function—cancer's Achilles' heel

Iron is an essential nutrient that plays an important role in almost all cellular processes, making it critical for virtually all cells [13]. Primarily, iron functions as a co-factor, or it is a constituent of co-factors, within the active sites of numerous proteins and enzymes (*e.g.*, hemoproteins and iron–sulphur cluster proteins) that play roles in cellular energy metabolism, DNA synthesis, cell growth and proliferation [7,13]. Indeed, iron is crucially involved in cellular respiration and energy transduction *via* oxidative phosphorylation and oxygen transport [14]. However, the molecular utility of iron's promiscuous redox activity means that high and/or improperly sequestered iron can result in cellular toxicity, predominantly resulting in the production of reactive oxygen species (ROS) that cause cellular dysfunction [14]. Consequently, it is important to monitor and maintain iron levels, in order to regulate normal cellular function and evade iron overload or deficiency.

2.1. Iron and ribonucleotide reductase

The necessity for a constant source of cellular iron is exemplified by the iron-dependence of the enzyme, ribonucleotide reductase (RR). Notably, RR is the rate-limiting enzyme for the *de novo* synthesis of all four 2′-deoxyribonucleotides from their 5′-ribonucleotide counterparts that are required as precursors for DNA synthesis and repair [6]. In addition to decreasing cellular iron uptake and increasing cellular iron mobilisation, this activity has long been believed to be the primary target of iron chelators in inhibiting cell growth and exerting their anti-cancer activity [15]. As with other higher organisms, mammals express only class Ia RR, which is a tetrameric enzyme ($\alpha_2\beta_2$) consisting of two homodimeric subunits, R1 and R2. R1 is the larger of the two subunits and contains the active sites enabling it to bind and reduce 5′-ribonucleotide substrates, while the R2 subunit has a dimeric iron binding site in each polypeptide chain [6,16]. In the active form, the R2 proteins contain a stable tyrosyl radical close to the iron centres, which occur in a high-spin ferric state and are anti-ferromagnetically coupled through a μ-oxo bridge [6]. The bound iron ions in each subunit of R2 redox cycle to abstract the hydrogen atom from the phenolic OH of a vicinal tyrosine to produce a tyrosyl radical that is essential for the catalytic activity of R1 [6]. In the absence of iron-binding to R2, the production of 2′-deoxyribonucleotides is inhibited [6]. Therefore, iron depletion potently inhibits RR activity and consequently inhibits DNA synthesis, ultimately leading to cell cycle arrest and apoptosis [17]. Specifically, the siRNA-mediated knockdown of R2 in HCT-116 human colon cancer