

Low hypoxia inducible factor-1 α (HIF-1 α) expression in testicular germ cell tumors – a major reason for enhanced chemosensitivity?

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Abstract

The molecular basis for enhanced chemosensitivity of testicular germ cell tumors (GCT) has been an area of great interest, as it could potentially give us therapeutic leads in other resistant malignancies. Thus far, however, the increased sensitivity of GCT has been variously attributed to multiple factors — an inability to detoxify cisplatin, a lack of export pumps, an inability to repair the DNA damage, an intact apoptotic cascade and lack of p53 mutation; but a unifying underlying etiology leading to the aforementioned processes and having a translational implication has so far been elusive. Herein, we offer evidence to support a potential significant role for the previously demonstrated low hypoxia inducible factor-1 α (HIF-1 α) expression in mediating the general exquisite chemosensitivity of testicular GCT, through the aforementioned processes. This molecular mechanism based hypothesis could have a significant translational implication in platinum refractory GCT as well as other platinum resistant malignancies.

Keywords: Hypoxia inducible factor-1 α (HIF-1 α); testicular germ cell tumor; chemosensitivity

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Introduction

When Vranic *et al.* discovered a low hypoxia inducible factor-1 α (HIF-1 α) expression in testicular germ cell tumors (GCT), specifically seminomas and mixed GCT (1), they may have accidentally stumbled upon an important underlying factor for the exquisite chemosensitivity of these tumors. Indeed, the molecular basis for enhanced chemosensitivity of GCT has been an area of great interest, as it could potentially give us therapeutic leads in other resistant malignancies. Thus far, however, the increased sensitivity of GCT has been variously attributed to multiple factors, — an inability to detoxify cisplatin, a lack of export pumps, an inability to repair the DNA damage, an intact apoptotic cascade and lack of p53 mutation (2-4).

Although the immediate cause for several of these factors has been identified [for example, low intracellular reduced glutathione (5) and metallothionein (6) leading to reduced conjugation and detoxification of cisplatin; and low expression of drug transport pumps such as lung resistance protein (LRP) (7) resulting in higher intracellular retention of chemotherapeutic agents], the root cause of these factors has not been delineated (for example, the cause of low intracellular reduced glutathione or the low expression of LRP in GCT has been unknown). We believe that the demonstrated low HIF-1 α expression in GCT may be the root cause for several of these noted chemotherapy sensitizing mechanisms, and have put forth postulated pathophysiologic pathways by which this may occur (Figure 1).

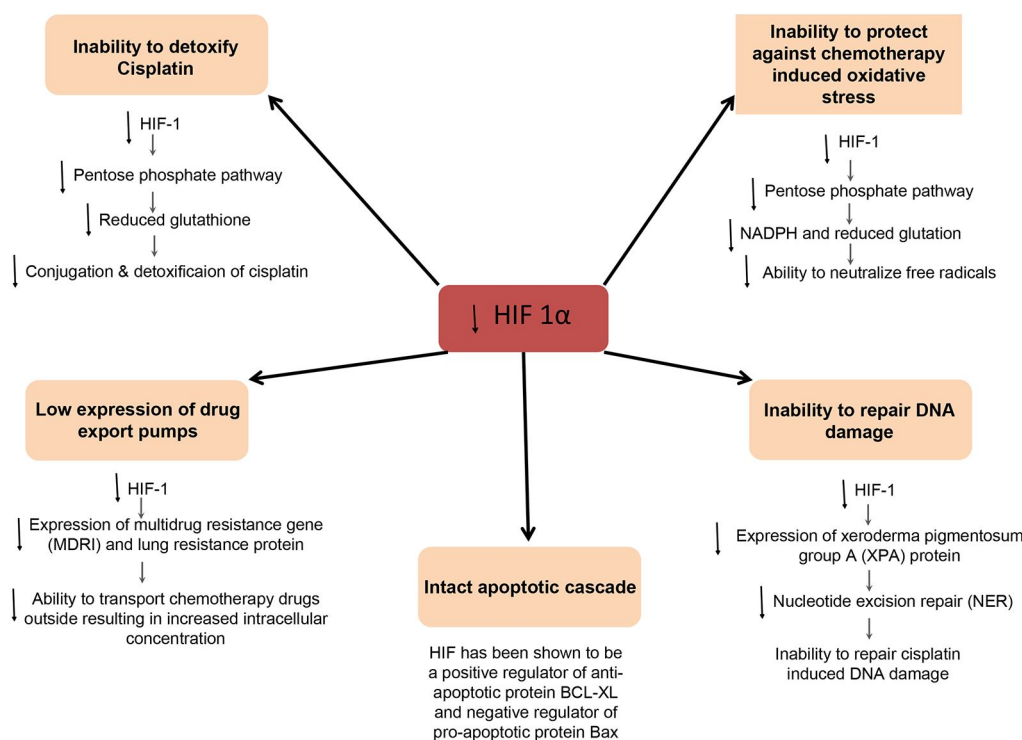


Figure 1 Potential central role of low hypoxia inducible factor-1 α (HIF-1 α) expression in the chemosensitivity of testicular germ cell tumors.

Inability to detoxify cisplatin

HIF-1 α increases uptake of glucose and mediates the diversion of glucose 6 phosphate into the pentose phosphate pathway, which produces increased amounts of NADPH and reduced glutathione (8,9). Low HIF-1 α may therefore be the reason for the low reduced glutathione found in testicular GCT, leading to reduced conjugation and detoxification of cisplatin.

Inability to protect against chemotherapy-induced oxidative stress

Reduced glutathione and NADPH generated through the pentose phosphate pathway are major defense mechanisms against the oxidative stress induced by chemotherapeutic agents. As such, tumor cells with low HIF-1 α would be expected to be vulnerable to chemotherapy induced oxidative stress.

Low expression of drug transport pumps

The expression of multidrug resistance 1 (MDR1) and

LRP, involved in drug transport and efflux, has been found to be synchronous with the changes in expression of HIF-1 α , and increased expression of these genes has been observed upon transfection with a plasmid HIF-1 α (10,11). These data suggest that HIF-1 α is a key regulator of MDR1 and LRP, involved in drug transport and efflux. LRP expression has been shown to be low in GCT (7), possibly due to low HIF-1 α levels.

Inability to repair DNA damage

One of the prominent reasons for the enhanced sensitivity of GCT to chemotherapy, specially cisplatin, is believed to be low levels of xeroderma pigmentosum group A (XPA) protein, a critical component of the nucleotide excision repair (NER) pathway (12). NER is the main mechanism by which DNA damage induced by cisplatin, in the form of bulky DNA adducts, is repaired (13). Binding of XPA to replication protein A (RPA) is the initiating, rate-limiting step of NER and it subsequently recruits other factors (14,15). Furthermore, increased expression of XPA has been associated with cisplatin resistance in other solid

tumors (16,17). Interestingly, HIF-1 binds to hypoxia response element (HRE) in the XPA promoter, and has been shown to up-regulate XPA, leading to cisplatin resistance in lung cancer. Furthermore, inhibition of HIF-1 α with siRNA has been shown to decrease the expression of XPA. Low HIF-1 α in GCT thus represents a potential cause of low XPA levels, in turn leading to an innate inability to repair DNA damage induced by cisplatin.

Intact apoptotic pathway

HIF-1 α is known to play a key role in regulating apoptosis resistance in tumor cells under hypoxia (18-20). GCTs have been shown to have low anti-apoptotic protein levels (BCL-2 and BCL-XL) (4) and HIF-1 α has been shown to be a key positive regulator of BCL-XL (19) and a negative regulator of pro-apoptotic protein Bax (21).

HIF-1 (a heterodimeric transcription factor consisting of the constitutively expressed HIF-1 β subunit and the oxygen-regulated HIF-1 α subunit) is known to confer worse prognosis in multiple cancers, with prominent effects on angiogenesis, cell survival and glucose metabolism (22); and mediates chemoresistance and radioresistance. PX-478, an inhibitor of HIF-1 α , has been shown to enhance radiosensitivity of various cancer cell lines and xenografts (23,24). Studies have also demonstrated reversal of chemoresistance with the use of small-interfering RNAs directed against HIF-1 α or with HIF-1 α destabilization (25,26). HIF-1 can up-regulate MDR gene expression and increased MDR gene expression has been shown to confer resistance to various drugs such as cisplatin, vinca alkaloids, anthracyclines, taxanes and epipodophyllotoxins (27,28). HIF-1 inhibition has been shown to down-regulate MDR1 expression (27). It is also a well-established fact that the hypoxic areas of cancers in general tend to be more radioresistant and chemoresistant, mediated by HIF-1 (29). The innate chemoresistance and radioresistance of renal cell carcinoma is thought to be caused, to a significant extent, by a constitutionally active HIF pathway due to loss of von Hippel-Lindau (VHL) function or accumulation of Krebs cycle intermediates (30-33). Pancreatic cancer, the most hypoxic of all solid tumors, remains refractory to a large extent to chemoradiotherapy with nuclear HIF-1 α in 88% of human pancreatic ductal carcinoma (34). HIF-1 therefore has a prominent role in mediating resistance in many cancers, and the development of direct or indirect HIF-1 inhibitors [summarized by Semenza *et al.* (22) and Onnis *et al.* (35)] continue to hold promise in enhancing

chemosensitivity and radiosensitivity in resistant malignancies. Clinical trials targeting HIF-1 in cancer are underway (ClinicalTrials.gov Identifier: NCT02564614 and NCT01120288).

Conclusions

Considering the above factors and the correlative evidence, a low level of HIF-1 may be a prominent underlying reason for the enhanced chemosensitivity of GCT. The unanswered questions that then arise are: what causes low HIF-1 in testicular GCT? Is it p53 induced degradation (36), with GCT rarely demonstrating inactivating mutation of p53, in contrast to other solid tumors (2)? If so, what explains the low p53 mutation incidence in testicular GCT? How is low HIF-1 related to isochromosome 12p, a common feature of testicular GCT?

Statement of translational relevance

Herein, we offer a molecular biology based hypothesis to support a potential significant role for the previously demonstrated low HIF-1 expression in mediating the general exquisite chemosensitivity of testicular GCT. Going forward, we plan to determine the difference in modulation of HIF-1 in platinum-resistant and sensitive GCT, and correlate it with patient outcomes.

If validated, this hypothesis could have a significant translational impact on platinum refractory GCT with the addition of HIF-1 modifying strategies to conventional chemotherapy regimens.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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