

Targeting Iron in Cancer Cells: A New Strategy to Inhibit Metastatic Progression

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Recent efforts to develop novel strategies for the treatment of cancer have focused on targeting the essential element, iron, which is crucial for DNA synthesis, cell cycle progression and metabolism [1]. This strategy led to the development of a potent and selective new class of anti-cancer agents known as the thiosemicarbazone iron chelators [1]. One of the most well characterized thiosemicarbazones is 3-AP or Triapine[®], which has entered over 20 Phase I and Phase II clinical trials [2-4]. Although 3-AP has shown some promise [2,5], its further development has been hampered by a host of serious side effects including hypoxia and methemoglobinemia [3,4]. To overcome these problems, a new series of dipyriddy thiosemicarbazone (DpT) iron chelators have been developed, with the lead agents being di-2-pyridylketone 4, 4-dimethyl-3-thiosemicarbazone (Dp44mT) and di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) [4]. These novel compounds were demonstrated to selectively inhibit tumor growth in a wide range of cancers both *in vitro* and *in vivo*, being well tolerated and having no marked toxicity at the optimal doses required for tumor inhibition [6,7]. These agents are also able to target a number of crucial molecules that control cancer progression including ribonucleotide reductase [8], p53 [9], cyclin D1 [6], p21 [6] etc. Furthermore, DpC does not induce metHb *in vivo* in animal models, and thus, this agent shows considerable advantages over both 3-AP and an earlier DpT analog, Dp44mT [10].

Although these studies present a significant step forward towards developing an effective treatment strategy for cancer, an important question still must be answered: can these agents inhibit cancer metastasis? To address this, a number of recent studies have examined one of the key down-stream targets of iron chelators, namely the metastasis suppressor, *N-myc down-stream regulated gene 1* (NDRG1) [6,7,11]. NDRG1 has been demonstrated to potently inhibit metastasis in a number of tumors including prostate, breast, lung, colon and pancreatic cancer, with its expression being correlated with less aggressive neoplasms and better patient prognosis [11-13]. Although the molecular mechanisms behind these effects remain to be completely established, there is increasing evidence that NDRG1 can inhibit the key pathways that are involved in metastasis, namely the transforming growth factor- β (TGF- β), phosphatidylinositol 3-kinase (PI3K), Ras and WNT signaling pathways [13,14].

TGF- β normally activates a signaling cascade that regulates cell proliferation [15]. However, in many cancer cells, this pathway is de-regulated due to the loss of a crucial down-stream target, namely the tumor suppressor, SMAD4 [15]. As a result, TGF- β becomes oncogenic, leading to the activation of Ras and PI3K pathways that promote cancer progression and metastasis (Figure 1; [15-17]). In fact, TGF- β can initiate epithelial to mesenchymal transition (EMT) in advanced cancer cells, leading to reduced membrane expression of the adhesion molecules E-cadherin and β -catenin, as well as increased cell migration and invasion [16]. Recent studies have revealed that NDRG1 can inhibit the oncogenic effects of TGF- β in cancer cells [14,16]. In fact, over-expression of NDRG1 increased the levels of E-cadherin and β -catenin at the membrane and inhibited both migration and invasion of colon and prostate cancer cells upon TGF- β treatment (Figure 1).

Moreover, NDRG1 also prevented the oncogenic down-stream effects of TGF- β in pancreatic cancer cells and this was mediated by an

increase in the key tumor suppressor molecules phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and SMAD4 (Figure 1; [14]). As a result, NDRG1 over-expression led to the inactivation of the oncogenic PI3K and Ras pathways [14,16].

Further studies examining the mechanisms behind the effect of NDRG1 on β -catenin expression revealed that this metastasis suppressor is also able to inhibit the WNT signaling pathway [13,16]. WNT signaling plays a crucial role in EMT and metastasis, leading to down-regulation of E-cadherin and an increase in oncogenic molecules such as cyclin D1 and Myc (Figure 1; [18]). The down-stream effects of WNT signaling are primarily mediated by β -catenin, which translocates from the membrane to the nucleus where it can function as part of a transcriptional complex with Lef-1 to mediate expression of its target genes (Figure 1; [18,19]). Recent studies have demonstrated that NDRG1 can inhibit WNT signaling by directly binding to the WNT co-receptor LRP6 [13]. In addition, NDRG1 also activates molecules such as GSK3 β , that are involved in degrading free β -catenin that is not part of the adherens junction (Figure 1; [13]). Hence, the combined effects of NDRG1 on the TGF- β , PI3K, Ras and WNT signaling pathways leads to increased levels of E-cadherin and β -catenin at the cell membrane, where they promote cell adhesion and inhibit cell invasion and metastasis.

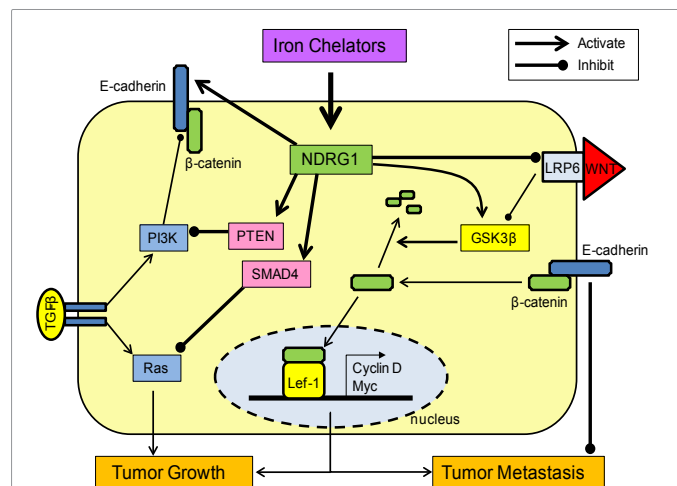


Figure 1: Schematic diagram of the molecular mechanisms by which NDRG1 inhibits tumor growth and metastasis.

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Considering the potent ability of NDRG1 to inhibit metastatic progression, targeting this latter molecule may be a promising strategy for cancer treatment. In fact, recent studies examining novel thiosemicarbazone iron chelators that markedly up-regulate NDRG1 expression in cancer cells have shown these agents to be highly effective at inhibiting cancer progression [6,7,13]. The mechanism involved in the up-regulation of NDRG1 involves hypoxia-inducible factor-1 α (HIF-1 α)-dependent and independent pathways [20]. The thiosemicarbazones, Dp44mT and DpC, were found to markedly up-regulate NDRG1 expression in pancreatic cancer cells, and this probably played a role in significantly reducing tumor growth *in vivo* [6]. Indeed, these latter agents also inhibited important down-stream targets of the Ras pathway, namely pERK and pSMAD2L *in vivo*, both of which are also down-regulated by NDRG1 [14].

In addition, Dp44mT and the “gold standard” iron chelator, desferrioxamine, also markedly up-regulated the cell adhesion markers, E-cadherin and β -catenin, in prostate and colon cancer cells, leading to reduced migration and invasion [16]. This was accompanied by a marked decrease in the mesenchymal marker, vimentin, further indicating that these agents are able to reverse the EMT [16]. Finally, Dp44mT was also demonstrated to inhibit breast cancer metastasis *in vivo*, with this effect being dependent on its ability to up-regulate NDRG1 [13].

In summary, the ability of novel thiosemicarbazone iron chelators to markedly inhibit cancer progression extends to more than just an inhibition of primary tumour growth. Indeed, these agents are also able to markedly reduce cancer metastasis and this is likely to occur, at least in part, through the up-regulation of the metastasis suppressor, NDRG1 (Figure 1). The ability of thiosemicarbazones to target NDRG1 and other key molecular players (e.g., cyclin D1, ribonucleotide reductase, p21, *etc.*) *via* their ability to bind iron demonstrates the potential of this exciting new group of anti-cancer agents.

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