NDRG1: a novel therapeutic target against metastatic cancers

Abstract
Tumor metastasis is a critical clinical problem that requires urgent attention. Recently, there has been growing interest in the development of metastasis suppressor proteins as targets for anti-cancer therapy. N-myc downstream regulated protein 1 (NDRG1) is an important and well-established metastasis suppressor protein that has shown promise as a significant therapeutic target to inhibit cancer metastasis. Notably, a novel series of thiosemicarbazone-based anti-cancer agents under development have shown the ability to up-regulate NDRG1. Therefore, further studies examining the effect of NDRG1 as a therapeutic target to prevent cancer metastasis are warranted.

Cancer metastasis
Cancer metastasis is the process by which tumor cells spread from the primary tumor to distant locations. It is one of the major clinical problems and accounts for about 90% of cancer-related deaths. Metastasis is a complex process and involves multiple steps.

i. Local invasion-Cells undergo epithelial-mesenchymal transition (EMT) and locally invades through the basement membrane.

ii. Intravasation-Tumor cells actively transverse through the walls of capillaries and lymphatics into the circulation.

iii. Transport-Cancer cells are transported to different sites, and if they survive the hostile conditions during transport, they adhere to solid supporting tissue and form micro-thrombi.

iv. Extravasation-Cancer cell micro-thrombi invade into distant tissue, typically lungs, brain, bone or liver.

v. Formation of micro-metastasis-After extravasation, cells lodge at secondary sites, where they must proliferate and colonize for successful metastasis. These metastatic processes are controlled by number of promoters and suppressors, which orchestrate a complex array of events to achieve successful metastasis. Recently, there has been growing interest in the role of metastasis suppressor genes, which have been shown to inhibit the formation of successful metastasis. To date, a number of metastasis suppressors have been identified, such as NDRG1, SSeCKs, KAI1 etc. These genes are generally found to be suppressed in advanced tumors and form an attractive target for the development of novel therapeutic agents.

NDRG1: A novel target against cancer metastasis
The NDRG1 (also known as RTP, Drg1, cap43, rit42, PROXY-1) gene encodes the well-known metastasis suppressor protein, NDRG1. It belongs to the NDRG family of proteins, with NDRG-2, -3 and -4 forming the other members of the family. The gene encodes a 3kb mRNA, which translates a 43kDa protein. NDRG1 is known to be down-regulated in number of cancers, such as breast, prostate, ovarian, etc. In fact, studies have shown that there is an inverse relationship between the severity of the tumor and NDRG1 levels, whereby lower levels of NDRG1 were observed in bio-specimens from more advanced and aggressive tumor stages.

Although the exact mechanism through which NDRG1 exerts its anti-metastatic effects is still not completely understood, significant progress has been made in this direction in past few years. Interestingly, Chen et al. have shown that NDRG1 can inhibit EMT via its ability to modulate the tumor growth factor-β (TGF-β) pathways. In fact, NDRG1 increased the membrane expression of E-cadherin, which is an important protein required for cell-cell adhesion. NDRG1 was also shown to suppress the TGF-β-induced SMAD signaling pathway, which also plays a critical role in EMT.

The WNT/β-catenin pathway is another important known regulator of the EMT. Recent studies have shown that NDRG1 can inhibit WNT-dependent signaling, leading to the suppression of EMT. Collectively, these investigations indicate that inhibition of EMT by NDRG1 is one of the important mechanisms via which it can inhibit cancer cell metastasis. Cancer cell migration is another integral step in successful metastasis of cancer cells. Studies by Sun et al. demonstrated that NDRG1 can suppress the Rho-associated coiled-coiled containing protein kinase 1 (ROCK1)/phosphorylated myosin light chain 2 (pMLC2) pathway. In fact, ROCK1/pMLC2...
is involved in formation of actin stress fibers, which are required for cellular migration.\textsuperscript{13,14} Thus, inhibition of ROCK1/pMLC2 pathway by NDRG1 could directly lead to inhibition of cellular migration, and thus, metastasis.\textsuperscript{15}

Src is an important promoter of cancer metastasis, which is known to exert a variety of effects on the metastasis-invasion cascade through a number of downstream regulators.\textsuperscript{16} Significantly, it has been shown to down-regulate E-cadherin,\textsuperscript{17} which itself is known to be a metastasis suppressor.\textsuperscript{18} Moreover, Src is also known to increase cell migration via its downstream effector molecules, such as p130Cas.\textsuperscript{19} A recent study by Liu et al.\textsuperscript{20} demonstrated that NDRG1 can inhibit the pro-metastatic effects of Src, leading to a phenotype with reduced metastatic ability. Collectively, these studies indicate that NDRG1 interacts with number of critical molecules involved in cancer metastasis and thus, exerts a multi-faceted anti-metastatic effect. Further studies are required to completely understand how this metastasis suppressor interacts with other crucial players in the complex metastatic process.

\textbf{Thiosemicarbazone anti-cancer agents}

Importantly, a novel series of thiosemicarbazone anti-cancer agents have been demonstrated to up-regulate NDRG1.\textsuperscript{19} These agents are shown to act via a double punch mechanism, leading to:

\begin{enumerate}
  \item Chelation of essential metal ions, such as iron and copper.\textsuperscript{19-21}
  \item Formation of redox active iron and copper complexes.\textsuperscript{19-21}
\end{enumerate}

Importantly, these ligands have potent and selective anti-tumor activity, both in vitro and in vivo.\textsuperscript{22-24} The lead compound di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) has demonstrated potent anti-tumor activity via oral as well as i.v. administration in vivo. It has also been shown that DpC has a very safe drug profile at the optimal doses required for effective anti-tumor activity.\textsuperscript{22,23} Notably, DpC has been recently been commercialized and will enter clinical trials in 2015.

It has been shown that NDRG1 was required for the activity of these thiosemicarbazones.\textsuperscript{20} In fact, there was a marked decrease in the anti-metastatic activity of these agents in breast cancer cells which were silenced with NDRG1, compared to cells with normal levels of NDRG1.\textsuperscript{16} Notably, thiosemicarbazone anti-cancer agents have also been shown to mediate similar effects as mediated by NDRG1 on number of pathways involved in cancer metastasis such as the TGF-β-induced EMT\textsuperscript{15} ROCK1/pMLC2 mediated formation of stress fibers for cellular migration\textsuperscript{17} and Src-mediated pro-metastatic pathway.\textsuperscript{18}

\textbf{Future directions}

NDRG1 has shown promise for development as an anti-metastatic drug target. The advancement of thiosemicarbazones as potential clinical agents, via their ability to up-regulate NDRG1, has opened a new opportunity for establishment of novel anti-metastatic therapies. In summary, further research is required to comprehensively understand the mechanisms via which NDRG1 exert its metastatic suppressive effects.

\textbf{Acknowledgements}

D.R.R. thanks the National Health and Medical Research Council of Australia (NHMRC) for a Senior Principal Research Fellowship and Project Grants. S.S. thanks Sydney Medical School for an Early Career Research Grant. D.S.K. thanks the NHMRC for a Project Grant [1048972] and a NHMRC RD Wright Fellowship [1083057].

\textbf{Conflict of interest}

D.R.R. is a stakeholder in the companies, Oncochel Therapeutics LLC and Oncochel Therapeutics Pty Ltd., that are developing the thiosemicarbazone, DpC, for the treatment of cancer. D.R.R. also consults for Oncochel Therapeutics LLC and Pty Ltd.

\textbf{References}

1. Weinberg R. The biology of cancer. 1st ed. Garland science; 2007.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674.
3. Stafford LJ, Vaidya KS, Welch DR. Metastasis suppressors genes in cancer. Int J Biochem Cell Biol. 2008;40(5):874–891.
4. Bae DH, Jansson PJ, Huang ML, et al. The role of NDRG1 in the pathology and potential treatment of human cancers. J Clin Pathol. 2013;66(11):911–917.
5. Fang BA, Kovacevic Z, Park KC, et al. Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. Biochim Biophys Acta. 2014;1845(1):1–19.
6. Chen Z, Zhang D, Yue F, et al. The iron chelators Dp44mT and DFO inhibit TGF-β-induced epithelial-mesenchymal transition via up-regulation of N-Myc downstream-regulated gene 1 (NDRG1). J Biol Chem. 2012;287(21):17016–17028.
7. Van Roy F, Bers G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci. 2008;65(23):3756–3788.
8. Zavadil J, Bottinger EP. TGF-β and epithelial-to-mesenchymal transitions. Oncogene. 2005;24(37):5764–5774.
9. Heuberger J, Birchmeier W. Interplay of cadherin-mediated cell adhesion and canonical Wnt signaling. Cold Spring Harb Perspect Biol. 2010;2(2):a002915.
10. Jin R, Liu W, Menezes S, et al. The metastasis suppressor NDRG1 modulates the phosphorylation and nuclear translocation of Beta-catenin through mechanisms involving FRAT1 and PAK4. J Cell Sci. 2014;127(Pt 14):3116–3130.
11. Liu W, Xing F, Iizumi-Gairani M, et al. N-myc downstream regulated gene 1 modulates Wnt-β-catenin signalling and pleiotropically suppresses metastasis. EMBO Mol Med. 2012;4(2):93–108.
12. Sun J, Zhang D, Zheng Y, et al. Targeting the metastasis suppressor, NDRG1, using novel iron chelators: regulation of stress fibrous-mediated tumor cell migration via modulation of the ROCK1/pMLC2 signaling pathway. Mol Pharmacol. 2013;83(2):454–469.
13. Chaturvedi LS, Marsh HM, Basson MD. Role of RhoA and its effectors ROCK and mDia1 in the modulation of deformation-induced FAK, ERK, p38, and MLC motogenic signals in human Caco-2 intestinal epithelial cells. Am J Physiol Cell Physiol. 2011;301(5):C1224–1238.
14. Katoh K, Kano Y, Noda Y. Rho-associated kinase-dependent contraction of stress fibres and the organization of focal adhesions. J R Soc Interface. 2011;8(56):305–311.
15. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. Nat Rev Clin Oncol. 2009;6(10):587–595.
16. Nagathihalli NS, Merchant NB. Src-mediated regulation of E-cadherin and EMT in pancreatic cancer. Front Biosci (Landmark Ed). 2012;17:2059–2069.
NDRG1: a novel therapeutic target against metastatic cancers

17. Reynolds AB, Kanner SB, Bouton AH, et al. SRFChing for the substrates of Src. *Oncogene*. 2014;33(37):4537–4547.

18. Liu W, Yue F, Zheng M, et al. The proto-oncogene c-Src and its downstream signaling pathways are inhibited by the metastasis suppressor, NDRG1. *Oncotarget*. 2015;6(11):8851–8874.

19. Merlot AM, Kalinowski DS, Richardson DR. Novel chelators for cancer treatment: where are we now? *Antioxid Redox Signd*. 2013;18(8):973–1006.

20. Jansson PJ, Hawkins CL, Lovejoy DB, et al. The iron complex of Dp44mT is redox-active and induces hydroxyl radical formation: an EPR study. *J Inorg Biochem*. 2010;104(11):1224–1228.

21. Lovejoy DB, Jansson PJ, Brunk UT, et al. Antitumor activity of metal-chelating compound Dp44mT is mediated by formation of a redox-active copper complex that accumulates in lysosomes. *Cancer Res*. 2011;71(17):5871–5880.

22. Kovacevic Z, Chikhani S, Lovejoy DB, et al. Novel thiosemicarbazone iron chelators induce up-regulation and phosphorylation of the metastasis suppressor N-myc down-stream regulated gene 1: a new strategy for the treatment of pancreatic cancer. *Mol Pharmacol*. 2011;80(4):598–609.

23. Lovejoy DB, Sharp DM, Seebacher N, et al. Novel second-generation di-2-pyridylketone thiosemicarbazones show synergism with standard chemotherapeutics and demonstrate potent activity against lung cancer xenografts after oral and intravenous administration in vivo. *J Med Chem*. 2012;55(16):7230–7244.

24. Yuan J, Lovejoy DB, Richardson DR. Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: in vitro and in vivo assessment. *Blood*. 2004;104(5):1450–1458.

Citation: Sahni S, Merlot A, Jansson P, et al. NDRG1: a novel therapeutic target against metastatic cancers. *Int Clin Pathol J*. 2015;1(1):1–3. DOI: 10.15406/icpj.2015.01.00001