Epigenetic therapy for cisplatin resistance in non-small-cell lung cancer: the way forward?

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Lung cancer, in particular non-small-cell lung cancer (NSCLC), is the most common cause of cancer-related death in the world [1]. Rates of lung cancer will continue to rise owing to high levels of cigarette consumption in Asia and the developing world, and aging populations. Despite increased surgical resection rates, optimization of chemoradiotherapy and the introduction of targeted therapies, the treatment outcome for NSCLC remains poor [1]. The current standard of care for the majority of patients suitable for therapy involves treatment with a platinum-based chemotherapy regimen [1]. However, only a proportion of patients benefit and the remainder develop resistance to platinum-based therapy. Ongoing research aims to understand both the molecular mechanisms underpinning this resistance and identify agents or therapies that could sensitize or even resensitize patients to cisplatin-based therapeutic regimens. Targeting epigenetic mechanisms may represent one such approach.

At its simplest, epigenetics can be described as stable and heritable changes in gene expression that are not due to changes

in the primary DNA sequence, and is most commonly used to describe chromatin-based regulation of gene expression [1,2]. The main mechanisms used involve the laying down of modifications to DNA and histones, that are then utilized by various chromatin-modifying enzymes which can be categorized as 'readers', 'writers' and 'erasers' [2]. More recently, miRNAs, a form of noncoding RNA, have also been shown to affect epigenetic regulation by directly targeting the epigenetic machinery, leading them to be described as 'epi-miRNAs' [1]. Aberrant epigenetic regulation is now well established as a frequent event in NSCLC, with all known forms of epigenetic regulation playing a role [1]. This 'epigenetic landscape' can potentially be targeted and may have important implications for the management and treatment of NSCLC [1,2]. Critically, an emerging body of evidence now links some of these epigenetic events with cisplatin resistance.

Evidence for DNA methyltransferases & cisplatin resistance

Aberrant DNA CpG methylation is frequent in NSCLC and upregulation of the

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enzymes responsible (DNA methyltransferases) is associated with poor prognosis in NSCLC [1]. Loss of gene expression of a panel of genes, such as *CHK2*, *EMX2*, *HtrA3*, *IGFBP-3* and *CHFR*, and miRNAs, such as miR-200c, are associated with both resistance and poorer survival to cisplatin therapy [1]. Similarly, methylation analysis at the *IGFBP-3* promoters combined with immunohistochemistry for phospho-EGF receptor, phospho-IGF1 receptor and phospho-AKT has now been shown to have predictive value for response to cisplatin with an accuracy and precision of 0.84 and 0.9, respectively ($p = 0.0062$) [3]. Targeting DNA methyltransferases may, therefore, have additional utility in sensitizing or resensitizing NSCLC to cisplatin therapies.

“New studies involving ‘low-dose’ demethylating agents have demonstrated encouraging results both *in vitro* and *in vivo*.”

The DNA demethylating agents Vidaza® (5-azacytidine) and Dacogen® (5-aza-2-deoxycytidine) have been US FDA approved for the treatment of myelodysplastic syndrome. Traditionally, cytotoxicity is a significant issue associated with the use of these agents in solid tumors. However, the amount of these agents required to elicit DNA methylation changes is lower than that required to achieve maximal cytotoxicity. New studies involving ‘low-dose’ demethylating agents have demonstrated encouraging results both *in vitro* and *in vivo* [4]. A Phase I/II clinical trial in NSCLC demonstrated objective, durable responses in patients, with four out of 19 patients obtaining major objective responses to subsequent anticancer therapies given immediately after epigenetic therapy [5]. These encouraging results may have important practical applications for the future use of cisplatin-based regimens in the treatment of NSCLC.

Linking the chromatin remodeling machinery with cisplatin resistance

Lysine acetyltransferases (KATs) have also been implicated in NSCLC pathogenesis. High expression and mutation of various members of this family (*KAT3A* and *KAT13B*) have been observed in NSCLC [1,2]. Strong evidence now links several members of this family (*KAT2B*, *KAT3A*, *KAT5*, *KAT13B* and *KAT13D*) with mechanisms of cisplatin resistance in NSCLC cell lines. For instance, KAT5 forms a complex with

E2F1 in response to cisplatin that promotes the accumulation of ERCC1 and subsequent repair of cisplatin-induced DNA lesions [6]. Preclinical inhibitors targeting KAT5 have been developed (NU9056 and MG 149), but their potential for either sensitizing NSCLC to cisplatin or reversing cisplatin resistance requires investigation.

Previously, we outlined the evidence demonstrating significant alterations to histone deacetylase (HDAC) family members in NSCLC [1]. Generally, levels of class I HDACs appear to be elevated, whilst those of the class II HDACs are reduced [1]. Targeting the class I HDACs may, therefore, have a role in inducing sensitivity to cisplatin. A recent article by Tesei *et al.* demonstrates that derivatives of valproic acid, a HDAC inhibitor that targets both class I and II HDACs, with a high potency for class I HDACs, can sensitize NSCLC cell lines to cisplatin [7]. In a further study, low levels of HDAC6 were found to correlate with a poor postoperative survival for patients with NSCLC [1]. HDAC6 expression is also associated with cisplatin resistance, and knockdown or inhibition of HDAC6 activity enhanced cisplatin-induced DNA damage [8]. These observations suggest that patients whose tumors have reduced HDAC6 levels may benefit from cisplatin-based therapy. The potential predictive value of HDAC6 expression for sensitivity to cisplatin-based chemotherapy requires further evaluation.

Lysine methyltransferases (KMTs) are an increasingly studied family of candidate therapeutic targets for solid malignancies. One such member is EZH2 or KMT6. KMT6 is overexpressed in NSCLC with predictive and prognostic value [1,9–11]. Knockdown of KMT6 has been shown to inhibit NSCLC proliferation, migration and invasion [9,10]. Overexpression of KMT6 occurs in cisplatin-resistant cell lines and knockdown results in enhanced sensitivity to cisplatin [10]. A small molecule inhibitor of KMT6 exists, 3-deazaneplanocin A, but to our knowledge clinical trials of this agent have yet to be initiated. *In vitro* studies have, however, shown that this compound shows efficacy in inhibiting cellular proliferation and inducing apoptosis in NSCLC cell lines [12].

BRG1 and BRM, members of the SWI/SNF chromatin remodeling complex are lost in a proportion of NSCLC cell lines (30%) and tumors (10%) [1]. Loss of expression is associated with significantly worse survival following surgery. By contrast, knockdown of either BRG1 or

BRM1 has been found to enhance cellular sensitivity to cisplatin in a lung cancer cell line [13]. Therefore, in patients with loss of BRG1 and/or BRM, cisplatin-based chemotherapy may have therapeutic value.

miRNAs & cisplatin resistance in NSCLC

A significant body of evidence has linked aberrant miRNA expression with NSCLC. The pattern of expression may distinguish small-cell lung cancer from NSCLC, and adenocarcinoma from other histological subtypes of NSCLC. miRNAs may also be biomarkers for diagnosis and prognosis of NSCLC, and may play a role in NSCLC tumorigenesis [1]. Loss of miR-200c, a key regulator of epithelial–mesenchymal transition, due to DNA CpG methylation occurs in NSCLC, while re-expression of this miRNA restored sensitivity to cisplatin [1]. Another miRNA that has been linked to both epigenetic mechanisms and cisplatin in lung cancer is miR-34a. This miRNA has been shown to regulate the HDAC, SIRT1, and cells pretransfected with miR-34a could increase the sensitivity of lung cancer cell lines to cisplatin [14]. However, in an analysis of patients who participated in a trial of radically resected NSCLC randomized to receive adjuvant cisplatin-based chemotherapy versus follow-up only, expression of miR-34a was neither predictive nor prognostic of survival [15]. The role of this miRNA as a predictive marker will require studies in the neoadjuvant setting.

The cancer stem cell hypothesis & drug resistance

Cancer stem cells (CSCs) are an area of intense research and NSCLC can exhibit stem-like features. Critically, the CSC-like subpopulations are associated with escape from cisplatin therapy [16]. Increasing evidence links epigenetic regulation to central elements of CSC biology [17]. As such, epigenetic targeting agents could potentially be used to target this subpopulation of cisplatin-resistant cells. An early indication for the potential utility of this strategy came

from the seminal work in lung cancer cells by Sharma *et al.*, whereby chromatin-modifying agents could sensitize ‘drug-tolerant persisters’ with stem-like markers (CD133⁺) to therapy, including cisplatin [18]. In this article a lysine demethylase (KDM), KDM5A, was identified as a critical element in the development of drug-tolerant persisters. Other KDMs overexpressed in NSCLC include KDM1A and KDM5B [1,19], and knockdown or inhibition of KDM1A suppresses proliferation, migration and invasiveness of NSCLC cell lines [19]. Inhibitors of KDM1A have been identified, including pargyline and Parnate[®]/tranlycypromine, and a series of novel inhibitors have also been developed that selectively target cancer cells with a stem cell phenotype [20], indicating the therapeutic potential for treating CSCs in NSCLC.

Conclusion

There is accumulating evidence to suggest that the epigenetic regulatory machinery may represent a novel area for study in relation to both identifying patients who will benefit from cisplatin-based therapeutic regimens, and to potentially resensitize patients to platinum-based chemotherapy. Furthermore, epigenetic therapies may play important roles in targeting the CSCs, which are thought to allow tumors to evade cisplatin, and as such may greatly enhance NSCLC patient outcomes. More work will be required to determine if such strategies will be effective, however, the recent data from Juergens *et al.* [5], is encouraging.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- O’Byrne KJ, Barr MP, Gray SG. The role of epigenetics in resistance to cisplatin chemotherapy in lung cancer. *Cancers* 3(1), 1426–1453 (2011).
- Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell* 150(1), 12–27 (2012).
- Cortés-Sempere M, De Miguel MP, Pernía O *et al.* IGFBP-3 methylation-derived deficiency mediates the resistance to cisplatin through the activation of the IGFIR/Akt pathway in non-small cell lung cancer. *Oncogene* doi:10.1038/onc.2012.146 (2012) (Epub ahead of print).
- Tsai HC, Li H, Van Neste L *et al.* Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer Cell* 21(3), 430–446 (2012).
- Juergens RA, Wrangle J, Vendetti FP *et al.* Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. *Cancer Discov.* 1(7), 598–607 (2011).

- 6 Van Den Broeck A, Nissou D, Brambilla E, Eymen B, Gazzari S. Activation of a Tip60/E2F1/ERCC1 network in human lung adenocarcinoma cells exposed to cisplatin. *Carcinogenesis* 33(2), 320–325 (2012).
- 7 Tesei A, Briagliadori G, Carloni S *et al.* Organosulfur derivatives of the HDAC inhibitor valproic acid sensitize human lung cancer cell lines to apoptosis and to cisplatin cytotoxicity. *J. Cell. Physiol.* 227(10), 3389–3396 (2012).
- 8 Wang L, Xiang S, Williams KA *et al.* Depletion of HDAC6 enhances cisplatin-induced DNA damage and apoptosis in non-small cell lung cancer cells. *PLoS One* 7(9), e44265 (2012).
- 9 Huqun, Ishikawa R, Zhang J *et al.* Enhancer of zeste homolog 2 is a novel prognostic biomarker in nonsmall cell lung cancer. *Cancer* 118(6), 1599–1606 (2012).
- 10 Lv Y, Yuan C, Xiao X *et al.* The expression and significance of the enhancer of zeste homolog 2 in lung adenocarcinoma. *Oncol. Rep.* 28(1), 147–154 (2012).
- 11 Wan L, Li X, Shen H, Bai X. Quantitative analysis of EZH2 expression and its correlations with lung cancer patients' clinical pathological characteristics. *Clin. Transl. Oncol.* doi:10.1007/s12094-012-0897-9 (2012) (Epub ahead of print).
- 12 Kikuchi J, Takashina T, Kinoshita I *et al.* Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non-small cell lung cancer cells. *Lung Cancer* 78(2), 138–143 (2012).
- 13 Kothandapani A, Gopalakrishnan K, Kahali B, Reisman D, Patrick SM. Downregulation of SWI/SNF chromatin remodeling factor subunits modulates cisplatin cytotoxicity. *Exp. Cell Res.* 318(16), 1973–1986 (2012).
- 14 Wang X, Dong K, Gao P *et al.* MicroRNA-34a sensitizes lung cancer cell lines to DDP treatment independent of p53 status. *Cancer Biother. Radiopharm.* doi:10.1089/cbr.2012.1218 (2012) (Epub ahead of print).
- 15 Voortman J, Goto A, Mendiboure J *et al.* MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. *Cancer Res.* 70(21), 8288–8298 (2010).
- 16 Bertolini G, Roz L, Perego P *et al.* Highly tumorigenic lung cancer CD133⁺ cells display stem-like features and are spared by cisplatin treatment. *Proc. Natl Acad. Sci. USA* 106(38), 16281–16286 (2009).
- 17 Van Vlerken LE, Hurt EM, Hollingsworth RE. The role of epigenetic regulation in stem cell and cancer biology. *J. Mol. Med. (Berl.)* 90(7), 791–801 (2012).
- 18 Sharma SV, Lee DY, Li B *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 141(1), 69–80 (2010).
- 19 Lv T, Yuan D, Miao X *et al.* Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer. *PLoS One* 7(4), e35065 (2012).
- 20 Wang J, Lu F, Ren Q *et al.* Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties. *Cancer Res* 71 (23), 7238–7249 (2011).