Influence of DDP Chemotherapy on miRNA Expression

Subjects: Biochemistry & Molecular Biology

Contributor: Maria Konoshenko, Yuriy Lansukhay, Sergey Krasilnikov, Pavel Laktionov

Platinum-based chemotherapy, cisplatin (DDP) specifically, is the main strategy for treating lung cancer (LC). However, there is a lack of predictive drug-resistance markers, and there is increased interest in the development of a reliable and sensitive panel of markers for DDP chemotherapy-effectiveness prediction. MicroRNAs represent a perspective pool of markers for chemotherapy effectiveness.

Keywords: lung cancer ; non-small cell lung cancer ; cisplatin ; DDP ; chemotherapy ; chemosensitivity

1. Cisplatin and Lung Cancer Chemotherapy

The main method of lung cancer (LC) treatment is chemotherapy. Platinum-based drugs are the gold-standard first-line treatments (ASCO and NCCN). The use of platinum-based drugs (cisplatin and carboplatin), including in combination with other chemotherapy agents (taxanes, pemetrexed, and antimetabolites), can achieve an overall survival for patients of 8 to 10 months on average [1]. A large and randomized comparison of four co-therapy regimens for stage IIIb and IV LC (cisplatin and paclitaxel, cisplatin and gemcitabine, cisplatin and docetaxel, and carboplatin and paclitaxel), which are the most commonly used regimens in clinical practice, showed that none of the used regimens had advantages over the others. The overall survival for all the studied regimens was about 10 months, with a one-year survival rate of 34% [2]. Patients with stage III LC undergo chemoradiotherapy (sequentially or simultaneously). This chemotherapy regimen is based on the basic drugs of the platinum group or their combination with other chemotherapy drugs, i.e., paclitaxel, etoposide, and vinblastine. The median progression-free survival of patients treated with chemoradiotherapy is poor (approximately eight months), with a five-year survival rate of only 15% [3]. An option to improve the situation is to switch to other treatment regimens, or example, treatment using the immunotherapy drug durvalumab, which is an inhibitor of the PDL 1 ligand that mobilizes the effector link of the antitumor immune system in a tumor microenvironment [4] or treatment of EGFR-positive LC patients using a combination of chemotherapy and tyrosine kinase inhibitors (platina-based chemotherapy and osemertinib) [5]. These chemotherapy schemes can achieve longer remission. However, these treatments have some limitations: immunotherapy is not available in all countries [6] and only about 15% of the general population of NSCLC patients have mutations in the EGFR gene [6][7].

Thus, platinum-based chemotherapy continues to be the main strategy for treating lung cancer [1][8]. Cisplatin (DDP) is the most widely used chemotherapy agent; it is an alkylating agent that effects inter- and intra-strand DNA cross-links, leading to cell-cycle arrest. However, drug resistance can develop, resulting in further development of a tumor and side effects such as myelosuppression, drug nephritis, nausea, vomiting, hearing loss, and polyneuropathy, which significantly reduce a patient's quality of life [9]. Acquired chemoresistance during treatment is a major problem for clinicians and is a major cause of therapeutic failure [10]. Various mechanisms of tumor resistance to DDP have been described, and, most recently, these mechanisms have been classified as follows: (1) pre-target resistance (before cisplatin binds to DNA), (2) target resistance (directly associated with DNA-cisplatin adducts), (3) post-target resistance (associated with apoptosis caused by DDP-mediated DNA damage), and (4) off-target resistance (affecting molecular mechanisms that do not present obvious links to DDP-induced signals) [10]. Regardless of the resistance type, a tumor's loss of sensitivity to DDP leaves a very short period of time for therapy correction aimed at increased patient survival. Clinical outcomes in the treatment of LC patients could be significantly improved through the introduction of non-invasive biomarker assays to predict and monitor the effectiveness of therapy [11]. However, there is a lack of reliable predictive drug-resistance markers and an urgent need to develop reproducible and highly sensitive panels of predictive markers for DDP-effectiveness assessment. Knowing a tumor's response to cisplatin in advance would help clinicians, both before and during treatments, to select effective drugs and to adjust chemotherapy programs from one option to another in a timely manner. Efforts to identify such markers have primarily focused on the mechanisms underlying DDP resistance. DDP-resistance regulation represents a complicated network of many factors and signaling pathways. Obviously, a set of markers is needed to detect different types of tumors and, subsequently, to highlight the typical principal or driving aberrations specific to a particular tumor. MicroRNAs (miRNAs) could be promising candidate biomarkers for DDP resistance in LC, due to the

multiple mechanisms by which they regulate the expression, and vice versa, for different target genes. Fortunately, there is considerable evidence on the association of aberrant miRNAs expression with DDP resistance in tumor cells.

2. MicroRNAs and Lung Cancer Chemotherapy

Since miRNAs regulate of a wide spectrum of physiological and pathological processes in cells, they are secreted from cells and enter the extracellular medium and biological fluids [12]. MicroRNAs have been shown to be rather stable in biological fluids, including blood or bronchial lavage, in which they circulate in tight complexes with biopolymers or are packed in membrane-coated vesicles [13][14] for review. Cell-free miRNAs (cfmiRNAs) can be released from different tumor areas or tumor nodes, and, therefore, a cfmiRNA profile reflects a patient's generalized tumor phenotype. Considering the well-developed protocols for cfmiRNA isolation and evaluation of their sets and concentrations, cfmiRNAs could be promising diagnostic markers [15]. The availability of liquid media, such as blood, sputum, and saliva, and methods that do not require invasive procedures have provided an opportunity for using liquid biopsies in the diagnosis of cancers, including LC [16]. The correlation between changes in miRNA expression and tumor development during treatment (aggressiveness and chemoresistance) have prompted the development of miRNA diagnostic panels and the emergence of prognostic and predictive markers for monitoring cancer as well as the development of new strategic solutions for the treatment of platinum-resistant LC ([17][18][19] for review). In fact, miRNA dysregulation in LC and under LC chemotherapy is involved in the regulation of the genes crucial to chemoresistance development: DNA repair, apoptosis, cell-cycle regulation, epithelial–mesenchymal transition (EMT), hypoxia, autophagy, drug efflux, cancer stem-cells activation (CSCs), etc. [19][20][21].

Numerous studies have aimed at identifying the miRNAs that mediate DDP response by investigating miRNAs that induce resistance/sensitivity to DDP in tumor cells or through comparative analyses of miRNA expressions in chemo-resistant and chemo-sensitive samples (cell lines and the tissues or biofluids of DDP-resistant and -sensitive LC patients). However, there are fewer studies that have aimed at exploring miRNAs differentially expressed under DDP chemotherapy.

3. Influence of DDP Chemotherapy on miRNA Expression

DDP chemotherapy, both as a powerful anticancer treatment and as a strong stressful intervention in organisms, causes significant changes in miRNA expression in tumor cells, as well as in many different normal cell types. However, until now, limited attention has been given to the analysis of the effect of DDP chemotherapy on the expression of miRNAs (**Table 1**).

Table 1. The effect of DDP on miRNA level in LC samples.

No.	DDP R/DDP S miRNA	Downstream Regulated Target Gene, Main Function/Pathway	Methods	In DDP R vs. DDP S Samples	Effect of DDP on miRNA Level	↑ of miRNA Expression → Chemoresistance	↓ of miRNA Expression → Chemosensitivity	Model: R/S Cells; Mice Xenografts	Reference
1	miR-33b-3p	P21	luciferase assay, RT-PCR, Western blot		↓ in cells	↑ cell viability, proliferation, promoted G1/S transition, DNA-damage response	↓ cell viability, G1 arrest,	S: A549 R: A549/DDP	[22]
2	miR-425-3p	AKT1, autophagy	luciferase assay, qRT-PCR, Western blot	↑ in cells, cells exosomes	↑ in serum exosomes, cell exosomes	↑ cell viability, ↓ apoptosis in S cells	↓ cell viability, ↑ apoptosis in R cells	S: A549 R: A549/DDP	[23]
3	miR-3195				↑ in cells, cell exosomes			S: A549	
4	miR-3676-5p				↑ in cells, cell exosomes			S: A549	[24]
5	miR-4443				↑ in cells, cell exosomes			S: A549	

No.	DDP R/DDP S miRNA	Downstream Regulated Target Gene, Main Function/Pathway	Methods	In DDP R vs. DDP S Samples	Effect of DDP on miRNA Level	↑ of miRNA Expression → Chemoresistance	↓ of miRNA Expression → Chemosensitivity	Model: R/S Cells; Mice Xenografts	Reference
6	Let7 (let-7a,-7b,-7c,-7d,-7e, -7f,-7g,-7i)	LIN28A,B	luciferase assay, IHC, RT-PCR, Western blot	↓ in tissues, cells	↓ in cells	↓ cell viability in R cells	↑ cell viability in S cells	S: A549 R: A549/DDP	[25]
7	miR-29c	AKT2	RT-PCR	↓ in tissues	↑ in cells	↓ cell viability in S ↓ tumor volume, proliferation (ki-67,AKT2) in X	↑ cell viability	S: SPC-A-1, A549 X: A549	[26]
8	miR-32	TRIM29		↓ in plasma	↑ in plasma				[27]
9	miR-181a				↓ in cells	↑ percentage of A549 cells with a G0-G1 DNA content ↑ proteolytic maturation of caspase-9 and caspase-3 triggered by CDDP ↑ proapoptotic member of the Bcl-2 family Bax	No effect	S: A549	[10] [23]
10	miR-1244	Bax, MEF2D, cyclin D1, p53	qRT-PCR, Western blot		↓ in cells	↓ proliferation, ↑ apoptosis		S: A549, H522	[28]

Nevertheless, miRNAs deregulated by DDP therapy represent a pool of prospective biomarkers for the development of a co-therapy, because they may reflect the development of a secondary resistance to DDP, which, in turn, may be influenced by changes in miRNA-expression levels. Liquid-biopsy studies [23], are of special interest, because they allow continuous observation of changes in miRNA-expression levels during LC therapy. However, such studies are only at their starting point and require normal-tissue DDP-response filtering.

There are also some studies that have aimed to associate miRNA expression with simultaneous resistance/sensitivity to different chemotherapies, including DDP (**Table 2**). They include investigations of DDP and other platinum-based drugs, taxanes, cytostatic vincristine, and cetuximab (IgG1 against epidermal growth factor, **Table 2**). The results of such studies have indicated that miRNAs are involved in the regulation of drug resistance via both common and different drug mechanisms, including multidrug resistance.

Table 2. miRNA and resistance to DDP and other chemotherapies.

DDP—cisplatin; DDP-S miRNA—miRNA associated with DDP sensitivity; DDP-R miRNA—miRNA associated with DDP resistance; R—chemotherapy-resistant cell line; S—chemotherapy-sensitive cell line; X—mice xenograft based on LC cell lines.

No.	DDP R/DDP S miRNA	↑ of miRNA Expression	↓ of miRNA Expression	Model: (1) R/S Cells (2) Mice Xenografts	Drug	Reference
1	miR-181a	↑ migration, invasion, EMT in S	↓ migration, invasion, EMT in R cells	S: A549 R: A549/PTX, A549/DDP	DDP, paclitaxel	[29]
2	Let7f miR-29a	↓ cell viability in S, R		S: H2030 cells	DDP, carboplatin	[30]
3	miR-34c-3p	↓ cell viability, migration; ↑ apoptosis in cells ↓ tumor weight in X		S: A549, H1299 X: A549 mice	DDP, taxol	[31]
4	miR-137	↓ cell proliferation, migration, induced cell apoptosis, arrested cell cycle in G1 phase and reversed drug resistance in R cells; ↓ tumor volume, weight, VEGF (angiogenesis) in X	↑ cell growth, migration, cell survival, cell-cycle G1/S transition, resistance (CCK-8 assay) in S cells	S: A549 R: A549/CDDP X: A549/CDDP	DDP, paclitaxel	[32]
5	miR-200c	↓ cell viability, proliferation invasion, EMT; ↑ apoptosis		S: H1299, H596, and H522	DDP, cetuximab	[33]

No.	DDP R/DDP S miRNA	↑ of miRNA Expression	↓ of miRNA Expression	Model: (1) R/S Cells (2) Mice Xenografts	Drug	Reference
6	miR-202	↓ cell viability, IC50; ↑ apoptosis in S; ↓ tumor volume in X ↓ IC50 in S		S: NCI-H441, A549 X: A549	DDP Oxaliplatin, carboplatin	[26]
7	miR-216b	↓ IC50; ↓ tumor weight in X	↑ IC50	S: A549, PC9	DDP, carboplatin, oxaliplatin	[34]
8	miR-495	↓ cell viability, intracellular DDP accumulation in S, R	↑ cell viability	S: A549 R: A549/DDP	DDP, carboplatin, trans-/-diaminocyclohexaneoxalatoplatinum	[35]
9	miR-497	↓ cell viability, ↑ apoptosis in R	↑ cell viability in S	S: A549 R: A549/DDP	DDP, vincristine	[36]

DDP—cisplatin; DDP-S miRNA—miRNA associated with DDP sensitivity; DDP-R miRNA—miRNA associated with DDP resistance; R—chemotherapy-resistant cell line; S—chemotherapy-sensitive cell line; X—mice xenograft based on LC cell lines.

References

1. Fennell, D.A.; Summers, Y.; Cadranel, J.; Benepal, T.; Christoph, D.C.; Lal, R.; Das, M.; Maxwell, F.; Visseren-Grul, C.; Ferry, D. Cisplatin in the modern era: The backbone of first-line chemotherapy for non-small cell lung cancer. Cancer Treat. Rev. 2016, 44, 42–50.
2. Schiller, J.H.; Harrington, D.; Belani, C.P.; Langer, C.; Sandler, A.; Krook, J.; Zhu, J.; Johnson, D.H.; for the Eastern Cooperative Oncology Group. Comparison of Four Chemotherapy Regimens for Advanced Non–Small-Cell Lung Cancer. N. Engl. J. Med. 2002, 346, 92–98.
3. Butts, C.; Socinski, M.A.; Mitchell, P.L.; Thatcher, N.; Havel, L.; Krzakowski, M. Tecemotide (L-BLP25) versus placebo after chemoradiotherapy for stage III non-small-cell lung cancer (START): A randomised, double-blind, phase 3 trial. Lancet Oncol. 2014, 15, 59–68.
4. Spigel, D.R.; Faivre-Finn, C.; Gray, J.E. Five-year survival outcomes with durvalumab after chemoradiotherapy in unresectable stage III NSCLC: An update from the PACIFIC trial. J. Clin. Oncol. 2021, 39, 8511.
5. Wu, Y.L.; Tsuboi, M.; He, J.; John, T.; Grohe, C.; Majem, M.; Goldman, J.W.; Laktionov, K.; Kim, S.W.; Kato, T.; et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. N. Engl. J. Med. 2020, 383, 1711–1723.
6. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA A Cancer J. Clin. 2021, 71, 209–249.
7. Pennell, N.A.; Neal, J.W.; Chaft, J.E.; Azzoli, C.G.; Jänne, P.A.; Govindan, R.; Evans, T.L.; Costa, D.B.; Wakelee, H.A.; Heist, R.S.; et al. SELECT: A Phase II Trial of Adjuvant Erlotinib in Patients With Resected Epidermal Growth Factor Receptor-Mutant Non-Small-Cell Lung Cancer. J. Clin. Oncol. 2019, 37, 97–104.
8. NCCN Guidelines. 2020. Available online: https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1450 (accessed on 25 June 2020).
9. Dasari, S.; Tchounwou, B. Cisplatin in cancer therapy: Molecular mechanisms of action. Eur. J. Pharmacol. 2014, 740, 364–378.
10. Galluzzi, L.; Senovilla, L.; Vitale, I.; Michels, L.; Martins, I.; Kepp, O.; Castedo, M.; Kroemer, G. Molecular mechanisms of cisplatin resistance. Oncogene 2012, 31, 1869–1883.
11. Zandberga, E.; Kozirovskis, V.; Ābols, A.; Andrējeva, D.; Purkalne, G.; Linē, A. Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. Genes Chrom. Can. 2013, 52, 356–369.
12. Liang, H.; Gong, F.; Zhang, S.; Zhang, C.Y.; Zen, K.; Chen, X. The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids. Wiley Interdiscip. Rev. RNA 2014, 5, 285–300.
13. Weber, J.A.; Baxter, D.H.; Zhang, S.; Huang, D.Y.; Huang, K.H.; Lee, M.J.; Galas, D.J.; Wang, K. The microRNA spectrum in 12 body fluids. Clin. Chem. 2010, 56, 1733–1741.

14. Konoshenko, M.Y.; Lekchnov, E.A.; Vlassov, A.V.; Laktionov, P.P. Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. BioMed Res. Int. 2018, 2018, 8545347.

15. Bryzgunova, O.; Konoshenko, M.; Zaporozhchenko, I.; Yakovlev, A.; Laktionov, P. Isolation of Cell-Free miRNA from Biological Fluids: Influencing Factors and Methods. Diagnostics 2021, 11, 865.

16. Liang, W.; Zhao, Y.; Huang, W.; Liang, H.; Zeng, H.; He, J. Liquid biopsy for early stage lung cancer. J. Thorac. Dis. 2018, 10, S876–S881.

17. Fadejeva, I.; Olschewski, H.; Hrzenjak, A. MicroRNAs as regulators of cisplatin-resistance in non-small cell lung carcinomas. Oncotarget 2017, 8, 115754–115773.

18. Lu, J.; Zhan, Y.; Feng, J.; Luo, J.; Fan, S. MicroRNAs associated with therapy of non-small cell lung cancer. Int. J. Biol. Sci. 2018, 14, 390–397.

19. Iqbal, M.A.; Arora, S.; Prakasam, G.; Calin, G.A.; Syed, M.A. MicroRNA in lung cancer: Role, mechanisms, pathways and therapeutic relevance. Mol. Aspects Med. 2019, 70, 3–20.

20. Chen, Y.; Lu, L.; Feng, B.; Chen, Y.; Lu, L.; Feng, B.; Han, S.; Cui, S.; Chu, X.; Chen, L.; et al. Non-coding RNAs as emerging regulators of epithelial to mesenchymal transition in non-small cell lung cancer. Oncotarget 2017, 8, 36787–36799.

21. Fan, T.; Wang, W.; Zhang, B.; Xu, Y.; Chen, L.; Pan, S.; Hu, H.; Geng, Q. Regulatory mechanisms of microRNAs in lung cancer stem cells. SpringerPlus 2016, 15, 1762.

22. Xu, S.; Huang, H.; Chen, Y.N.; Deng, Y.T.; Zhang, B.; Xiong, X.D.; Yuan, Y.; Zhu, Y.; Huang, H.; Xie, L.; et al. DNA damage responsive miR-33b-3p promoted lung cancer cells survival and cisplatin resistance by targeting p21WAF1/CIP1. Cell Cycle 2016, 15, 2920–2930.

23. Ma, Y.; Yuwen, D.; Chen, J.; Zheng, B.; Gao, J.; Fan, M.; Xue, W.; Wang, Y.; Li, W.; Shu, Y.; et al. Exosomal Transfer Of Cisplatin-Induced miR-425-3p Confers Cisplatin Resistance In NSCLC Through Activating Autophagy. Int. J. Nanomed. 2019, 14, 8121–8132.

24. Qin, X.; Yu, S.; Zhou, L.; Shi, M.; Hu, Y.; Xu, X.; Shen, B.; Liu, S.; Yan, D.; Feng, J. Cisplatin-resistant lung cancer cell-derived exosomes increase cisplatin resistance of recipient cells in exosomal miR-100-5p-dependent manner. Int. J. Nanomed. 2017, 12, 3721–3733.

25. Yin, J.; Zhao, J.; Hu, W.; Yang, G.; Yu, H.; Wang, R.; Wang, L.; Zhang, G.; Fu, W.; Dai, L.; et al. Disturbance of the let-7/LIN28 double-negative feedback loop is associated with radio- and chemo-resistance in non-small cell lung cancer. PLoS ONE 2017, 12, e0172787.

26. Sun, W.; Ping, W.; Tian, Y.; Zou, W.; Liu, J.; Zu, Y. miR-202 Enhances the Anti-Tumor Effect of Cisplatin on Non-Small Cell Lung Cancer by Targeting the Ras/MAPK Pathway. Cell. Physiol. Biochem. 2018, 51, 2160–2171.

27. Xu, S.; Li, J.; Chen, L.; Guo, L.; Ye, M.; Wu, Y.; Ji, Q. Plasma miR-32 levels in non-small cell lung cancer patients receiving platinum-based chemotherapy can predict the effectiveness and prognosis of chemotherapy. Medicine 2019, 98, e17335.

28. Li, G.J.; Zhao, G.Q.; Yang, J.P.; Zhou, Y.C.; Yang, K.Y.; Lei, Y.J.; Huang, Y.C. Effect of miR-1244 on cisplatin-treated non-small cell lung cancer via MEF2D expression. Oncol. Rep. 2017, 37, 3475–3483.

29. Li, H.; Zhang, P.; Sun, X.; Sun, Y.; Shi, C.; Liu, H.; Liu, X. MicroRNA-181a regulates epithelial-mesenchymal transition by targeting PTEN in drug-resistant lung adenocarcinoma cells. Int. J. Oncol. 2015, 47, 1379–1392.

30. Yang, L.Z.; Lei, C.C.; Zhao, Y.P.; Sun, H.W.; Yu, Q.H.; Yang, E.J.; Zhan, X. MicroRNA-34c-3p target inhibiting NOTCH1 suppresses chemosensitivity and metastasis of non-small cell lung cancer. J. Int. Med. Res. 2020, 48, 300060520904847.

31. Yang, Z.; Liu, C.; Wu, H.; Xie, Y.; Gao, H.; Zhang, X. CSB affected on the sensitivity of lung cancer cells to platinum-based drugs through the global decrease of let-7 and miR-29. BMC Cancer 2019, 19, 948.

32. Shen, H.; Wang, L.; Ge, X.; Jiang, C.F.; Shi, Z.M.; Li, D.M.; Liu, W.T.; Yu, X.; Shu, Y.Q. MicroRNA-137 inhibits tumor growth and sensitizes chemosensitivity to paclitaxel and cisplatin in lung cancer. Oncotarget 2016, 7, 20728–20742.

33. Ceppi, P.; Mudduluru, G.; Kumarswamy, R.; Rapa, I.; Scagliotti, G.V.; Papotti, M.; Allgayer, H. Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in non-small cell lung cancer. Mol. Cancer Res. 2010, 8, 1207–1216.

34. Huang, G.; Pan, J.; Ye, Z.; Fang, B.; Cheng, W.; Cao, Z. Overexpression of miR-216b sensitizes NSCLC cells to cisplatin-induced apoptosis by targeting c-Jun. Oncotarget 2017, 8, 104206–104215.

35. Song, L.; Li, Y.; Li, W.; Wu, S.; Li, Z. miR-495 enhances the sensitivity of non-small cell lung cancer cells to platinum by modulation of copper-transporting P-type adenosine triphosphatase A (ATP7A). J. Cell. Biochem. 2014, 115, 1234–

1242.

36. Zhu, W.; Zhu, D.; Lu, S.; Wang, T.; Wang, J.; Jiang, B.; Shu, Y.; Liu, P. miR-497 modulates multidrug resistance of human cancer cell lines by targeting BCL2. Med. Oncol. 2012, 29, 384–391.