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Overcoming multiple drug resistance in lung cancer using siRNA targeted therapy

Sanaz Naghizadeh ¹, Ali Mohammadi ², Behzad Baradaran ¹, Behzad Mansoori ¹,²,³*

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

2. Department of Cancer and Inflammation Research, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark.

3. Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

Corresponding author: Behzad Mansoori, Ph.D., Immunology Research Center, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran. Phone: +98-41-33371440 Fax: +98-41-33371311, Email: Mansoorib@tbzmed.ac.ir
Abstract

Among cancers, lung cancer is the most morbidity and mortality disease that is remaining the fatalist. Generally, there are multiple treatment procedures for lung cancer, such as surgery, immunotherapy, radiotherapy and chemotherapy. There is, therefore, an urgent need for more specified and efficient methods for treatment of lung cancer such as RNAi, which in combination with traditional therapies could silence genes that are involved in the drug resistance. These genes may either be motivators of apoptosis inhibition, EMT and DNA repair system promoters or a member of intracellular signaling pathways, such as JAK/STAT, RAS/RAF/MEK, PI3K/AKT, NICD, β-catenin/TCF/LEF and their stimulator receptors including IGFR, EGFR, FGFR, VEGFR, CXCR4, MET, INTEGRINS, NOTCH1 and FRIZZLED, so could be considered as appropriate targets. In current review, the results of multiple studies which have employed drug application after one specific gene silencing or more than one gene from distinct pathways also simultaneous drug and RNAi usage in vitro and in vivo in lung cancer were summarized.

Key words: Drug resistance, Gene silencing, Lung cancer, RNA interference
1-Introduction

Genetic and epigenetic changes step by step by damaging DNA and altering normal proliferation and survival pathways in normal epithelial cells of lungs transform them into cancerous cells (1, 2). Lung cancer clinically, histologically, biologically and molecularly is a heterogeneous disease that is consisted of two major types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) that accounts for 15-20% of cases. NSCLC which constitutes for 80-85% of all diagnosed cases is more subdivided to large cell carcinoma, squamous cell carcinoma, adenocarcinoma, bronchoalveolar carcinoma and mixed ones such as adenosquamous carcinoma (2-5). Nowadays, NSCLC is further subcategorized by understanding molecular mechanisms and driver mutations of oncogenes (1, 3). Lung cancer remains globally dominant in terms of incidence and deadliest cancer among both genders (1, 6, 7). Therefore, it is predicted that approximately 2,093,876 new cases of lung cancer to be diagnosed and 1,761,007 deaths of this cancer occurs in 2018 globally (8). Symptoms of non-metastatic lung cancer are dyspnea, coughing, chest pain, however, for metastatic disease are anemia, weight loss, bone pain, headaches and so on. Also, preliminary diagnose is achievable with screening of the cancer via computed tomography scan (CT scan). Proved risk factors for lung cancer development are smoking, second-hand smoking, asbestos, and radon gas and so on. It is obvious that prevention of lung cancer diseases could be accessible by keeping away from the mentioned risks, especially smoking (9). Currently, available therapies for lung cancer are surgery, vascular endothelial growth factor (VEGF) inhibition, immunotherapy, stem cell inhibition, radiotherapy, chemotherapy and targeted therapy (10). Despite the existence of various treatments for lung cancer patients, 5-year survival rate is still lower than 20% (11).

Chemotherapy is a systematic treatment that is used to control primary and metastatic disease. It is also a general therapeutic method for the patients that molecular changes or driver mutations are not detected. Though resistance to multiple chemotherapeutic agents occurs and reduces the response rate of chemotherapy to only 20% to 35%, it is not effective enough for the patients bearing lung cancer (5, 10, 12, 13). Targeted therapy as an alternative treatment could compensate chemotherapy shortage at least in theory. As previously mentioned, finding driver mutations of oncogenes and discovery of tumor biology in a subset of lung cancers promotes
discovering specified targeted therapies against them. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) to inhibit mutated EGFR oncogene or Anaplastic lymphoma kinase (ALK) inhibitors targeting a fusion gene formed from ALK and Echinoderm microtubule-associated protein-like 4 (EML4) genes are examples of this specified targeted therapy (14). Likewise, unfortunately, resistance to target therapy follows within 1 year (5).

Cancer recurrence and metastasis after treatment, which is originated from chemotherapy or targeted therapy resistance, is the main cause of lung cancer therapy failure (15). Like other cancers, genetic and epigenetic changes by multiple mechanisms contribute to resistance against chemotherapy or targeted therapy in lung cancer. Regarding to the existing of several comprehensive reviews, containing explanations of Multiple drug resistance (MDR) mechanisms in lung cancer elaborately, we just tend to mention these mechanisms containing, secondary mutations of target genes, gene amplification, gatekeeper mutations, activation of downstream pathways of targets, the activation of receptors other than target receptors, bypass signal activation, apoptosis prevention, autophagy, gene overexpression, the abnormality of insulin-like growth factor 1 receptor (IGF1R), abnormality of molecules of multiple angiogenesis pathways, gene deletions, excess secretion of Interleukins (ILs), epithelial–mesenchymal transition (EMT), effusion of ATP-binding cassette (ABC) proteins, transmission of NSCLC to SCLC, expression of fusion genes, activation of DNA repair system, presence of Cancer stem cells (CSCs), tumor microenvironment and tumor heterogeneity (16-26).

According to the lack of response and failure of existing therapeutically procedures, it is obvious that there is an urgent need for more effective treatments to be well-tolerated, have the fewer side effects, provide better prognosis and eventually can be considered as an individualized therapy (27). Gene therapy includes these traits and nowadays is more attended for malignancies; therefore, it can be chosen as a proper approach (28).

Disruption of gene expression at both transcriptional and post-transcriptional stages is known as gene silencing. Among PTGS (post transcriptional gene silencing) methods, RNA interference (RNAi) application in experiments has been raised. Reduced toxicity, elevated specificity and effectiveness are some of the reasons of this increment. Long double-stranded RNAs and small interfering RNAs that may be synthetic or originate from viruses or transposons or short hairpin RNAs (shRNAs) are central elements of this process when introduced and expressed by plasmids
and microRNA (miRNAs). In any case, after entering cytoplasm and cleavage by DICER, they would be converted to 21-23 duplex fragments with 19 base pairs (bps) paired nt ds with 2-3 bp overhanging at 3’ ends. In cooperation with RNA-induced silencing complex (RISC) complex duplexes get unwound, the sense strand is degraded and antisense strand which is a complement to intended mRNA leads to breakage of target mRNA by Argonaute (AGO). Consequently, the objective gene is silenced (29). These silenced genes could be one of the genes, which are engaged in cell proliferation, apoptosis, survival, metastasis and genes or pathways that are responsible for the drug resistance (30-32).

Lack of selectivity and distribution specificity could lead to insufficient penetration to cancerous cells and reduced effectiveness of chemotherapeutical agents, on the other hand, increase the toxicity to other normal and healthy tissues and enhance MDR in tumors that are related to the ineffective response of the cancer cells to the chemotherapy agents (11, 31, 33). Reduced chances of side effects could decrease cytotoxicity, a high degree of specificity and efficacy, augment safety and potency, and also enhance stability/versatility. The possibilities of more easily and less expensively synthesizing are also advantages of Small interfering RNA (siRNA) usage (34-37). Besides these, a combination of siRNA with chemotherapy shows more advantages, such as the capability of protein expression haltering with high sequence selectivity before translation instead of sequestering protein, like targeted therapy agents (38). The combinational treatment is, therefore, associated with the increased synergistic effect of each therapeutic modality, resulting in a reduction of required doses of drugs, which yet are efficient sensitizes cancer cells to therapy several folds, enhancement of efficacy and at the end hinderance of the drug resistance and improvement of therapeutic outcomes for the patients. Because of these, a combination of RNAi-based therapy plus traditional drug-based treatments has been attracted a lot of attention (30, 31, 33, 39, 40).

There are many reports based on more efficiently overbearing drug resistance by chemotherapy and gene therapy combination. Here in this review, we tried to point out to the experiments that have been applied the combination of RNAi with chemotherapy for lung cancer treatment.
2-Invitro experiments displaying chemotherapy drug efficacy enhancement after RNAi application

2-1-drug application after gene silencing

That is an undeniable fact that a part of chemotherapeutic agents such as cisplatin functions via formation of DNA adducts and contribution to DNA damage, cell cycle arrest and apoptosis induction (41). Thus, it is sensible to assume that the overexpressed DNA damage repairing proteins and apoptosis inhibitors could resist cells against chemotherapeutic agents. Consequently, eradicating these proteins could reverse sensitization. For instance about DNA damage repairing proteins can mention to the relevance between Fanconi anaemia / breast cancer susceptibility protein (FA/BRCA) repair pathway and resistance to cisplatin has been indicated by some studies. Therefore, it could be supposed that targeting genes that are participated in this pathway may reverse cisplatin resistance. Fanconi anemia group F protein (FANCF) and Fanconi Anemia Complementation Group L (FANCL) depletion by siRNA potentiated cisplatin-resistant and sensitized A549 cells to cisplatin. Also, gene knockdown has synergized effect of cisplatin-induced apoptosis through inhibition of FA/BRCA pathway. Of course, this sensitization effect was more significant in A549/cisplatin-DDP (resistant) cells (42) (Fig.1). RAD51 is a critical protein for Homologous recombination (HR) and FA repair pathways. Enhanced level of RAD51 in NSCLC samples and its association with chemotherapy resistance was reported by Xiuli Chen and colleagues. They also showed that RAD51 downregulation by lentivirus could carry Rad51c short hairpin RNA on A549 cells that resulted in the elevated apoptotic cell death induced by cisplatin, and, the increment of sensitivity of A549 cells to cisplatin-mediated growth inhibition (43, 44) (Fig.1). Weiwei Wang and colleagues in a study used Excision Repair Cross-Complementation Group 1 (ERCC1) shRNA to silence ERCC1, a key role player in Nucleotide excision repair (NER) pathway. This suppression sensitized XWLC05 cells to ciplatin that could be proved by decreased half maximal inhibitory concentration (IC_{50}) of shRNA-infected cells to 1.34 μM versus 4.54 μM for the control cells. It was also shown that decreased cell proliferation, and increased apoptotic rate after cisplatin treatment were achieved (45, 46) (Fig.1). DNA topoisomerase 2-binding protein 1 (TOPBP1) acts as a cooperative of topoisomerase IIfβ (ataxia telangiectasia and Rad3-related protein (ATR) activator) that also interplays by several transcription factors such as P53. There are different reports stating inactivation of P53 by TOPBP1 overexpression. TOPBP1 down-regulation by specific siRNA in NCIH1299, human
l lung cancer cell line contributed to increased doxorubicin (Dox) sensitivity of the cells, on the other hand decreased Dox-induced P53 expression. According to the obtained result, it can be hypothesized that TOPBP1-dependant P53 induction by Dox leads to drug resistance (47).

Apoptosis process when is triggered by intrinsic signal, via P53 induction heads off Bel-2 homologous antagonist/killer (BAK) and BCL2-associated X protein (BAX) inhibition by B-cell lymphoma 2 (BCL2), B-cell lymphoma-extra-large (BCL-XL) and Myeloid Cell Leukemia 1 MCL1. Thus, BAK and BAX oligomerization forms pores in mitochondria membrane and subsequently cytochrome-c release. Eventually, cytochrome-c/ Apoptotic protease activating factor 1 (APAF1)/ caspase9 (C9)/ caspase3 (C3), caspase7 (C7) activation ends up in Poly (ADP-ribose) polymerase PARP cleavage and cell death (48) (Fig.1). About apoptosis pathway can remark to experiments done on BCL2 family members or other participating proteins. Proto-oncogene, BCL2 is an antiapoptotic protein that when is overexpressed delays onset of chemotherapy drug-induced apoptosis. In a study that was managed by Zexiang Huang and et al. it was observed that BCL2 expression was elevated in A549/DDP cells than A549 cells. They also showed that BCL2 inhibition by siRNA in A549/DDP cells could sensitize the cells to DDP and Diallyl disulfide(DADS) compared with control group (28) (fig.1). Also, BCL2 is an AKT downstream protein, Man Zou and colleagues examined impact of its knockdown by siRNA on gefitinib sensitization of H1975 lung cancer cell line. Downregulated BCL2 led to the induction of apoptosis, and moreover gefitinib addition to the transfected cells intensified apoptotic effect and reversed EGFR-TKIs-resistant trait of the considered cells (49) (Fig.2). BCL-XL, functional and structural homolog of BCL2, which is linked to malignancies protection against chemotherapeutic agents was downregulated by siRNA in a study done by Xiaoyong Lei. The results indicated that BCL-XL siRNA led to sensitization of A549/DDP cells to DDP and increased effectiveness of the drug also increased DDP-induced cell death. These gained results may be due to the induction of cytochrome c-mediated caspase-dependent apoptosis by BCL-XL siRNA (50) (Fig.1). Promotion of cisplatin resistance in lung cancer by Wingless/Integrated (WNT)/β-catenin pathway through BCL/XL enhancement has been also suggested. To examine this hypothesis, Jin Zhang and et al. applied β-catenin siRNA in A549 cells, afterwards, cisplatin-induced apoptosis was increased and cisplatin-induced growth inhibition was further reduced; however, BCL/XL expression was declined. Therefore, β-catenin silencing with siRNA
could be contributed to cisplatin sensitivity probably through BCL/XL down regulation (51) (Fig.2). IAPS (inhibitors of apoptosis) are a group of proteins which by binding to proapoptotic proteins block their functions. A well-known property of IAPS is BIR (baculovirus IAP receptor) domain. BIRC6 is a member of this family that is capable of binding to caspase3, 6, 7 and 9 to inactivate them. Xin Dong et al. managed a study to investigate BIRC6’s role in cisplatin resistance on NSCLC. First of all, they observed that BIRC6 was overexpressed in NSCLC clinical specimens and its elevated levels were associated with metastasis to lymph node. BIRC6 siRNA increased cisplatin-induced apoptosis of A549 lung adenocarcinoma cells, consequently, sensitized the cells to cisplatin compared with the controls (52) (Fig.1). Also, livin’s targeting (one of IAP family members, inhibitor of caspase-3, -7, and -9) by RNAi, sensitized NSCLC cells to etoposide chemotherapeutic agent and inhibited cell growth (53, 54) (Fig.1).

Furthermore, there are many other proteins from diverse signaling pathways that could stimulate resistance. Many attempts have been done to eliminate their expression, which mostly by increment of apoptosis and bring back sensitivity of the cells. For example, a siRNA pool, including EGFR specific siRNAs in combination with TKIs (gefitinib, erlotinib, afatinib) or cetuximab could rescue the sensitivity of T790M mutant H1975 cells to these chemotherapeutic agents since cell growth inhibition was enhanced and apoptosis was induced in siRNA plus drug combination group compared with the single agents (55) (Fig.2). Casein kinase 2 (CK2) is a ser/thr kinase, which inhibitsPromyelocytic leukemia protein (PML) as an apoptosis inducer. There is an inverse relation between CK2 and PML levels in NSCLC. Besides, Bo Yang and et al. in a study showed that cisplatin increased CK2A expression in A549 and H157 cells. The application of siRNA against CK2A increased apoptosis induction by cisplatin and decreased the cell viability. Cisplatin activates CK2 that in turn suppresses PML through P38/mitogen-activated protein kinase (MAPK) pathway. Consequently, it can be concluded that CK2 silencing may enhance cisplatin sensitivity through PML elevation (56) (Fig.2). In the recent years, there have been some reports based on Mediator complex subunit 19 (MED19) overexpression in NSCLC patients. MED19 is a component of head-module subunits of mediator complex. This complex is involved in RNA polymerase II–mediated transcription. Mediator complex has been suggested to be associated with Wnt/b-catenin signaling pathway. It was also detected that MED19 participates in direct regulation of Wnt/b-catenin path with an unclear mechanism. In
another experiment, MED19 siRNA was employed on NCSLC cell lines A549 and SPCA1 to assess relation between MED19 silencing and chemo-sensitivity to cisplatin. The results revealed that after MED19 depletion, cisplatin sensitivity was increased and apoptosis induction by cisplatin was promoted (57, 58). Pyruvate kinase isozymes M2 (PKM2), one of isoforms of pyruvate kinase enzymes, is expressed in adult stem cells, embryonic cells and tumor cells. PKM2 is a Phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K)/AKT/m-TOR downstream gene and its overexpression is verified in numerous cancers. In a research, Sujuan Yuan et al. tried to explore whether PKM2 silencing with method of plasmid transfection will affect on A549 and H460 cells’ sensitivity to docetaxel. Docetaxel is a chemotherapeutic agent which by inhibiting - microtubule disassembly, blocking cells at G2/M phase halts mitosis progression and promotes apoptosis. In this study, it was found that PKM2 knockdown with shRNA, caused cell viability inhibition stronger than docetaxel usage lonely, improved docetaxel sensitivity, its cytotoxic effect and also apoptosis induction effect of docetaxel on A549 and H460 cells (12, 59) (Fig.2). Enhancer of zeste homolog 2 (EZH2) is a methyltransferase and catalytic component of PRC2 complex that by DNA or histone methylation leads to gene inactivation containing tumor suppressor genes. This gene is stabilized by Wnt/β-catenin pathway. USP1 Transcription factor 4 (TCF4) interacts with and deubiquitinates EZH2 after getting induced by β-catenin/. Ubiquitin-degradation survived EZH2 triggers its activities. There are studies correlating EZH2 with drug resistance in some types of tumors. In a study carried out by Wen Zhou et al. EZH2 silencing by siRNA not only increased cisplatin sensitivity in A549/DDP cells significantly but also, elevated apoptosis rate after cisplatin treatment (60, 61) (fig.2). Chengyao Xie and colleagues could inhibit C-MYC expression by siRNA, and show that by C-MYC inhibition, the IC₅₀ value of cisplatin was reduced and apoptosis was increased. In addition, to analyze cisplatin and c-MYC siRNA co-treatment effect on cell cycle, three groups (cisplatin, c-myc siRNA, cisplatin plus C-MYC siRNA) were established and the results showed that the number of G0/G1 cells were increased in all groups. Nevertheless, co-treated group with cisplatin and siRNA exhibited the most changes. C-MYC as a downstream target gene of WNT/β-catenin pathway, and also PI3K/AKT pathway could augment c-MYC expression. One of C-MYC functions is regulation of cells existing from G1 phase and entering S phase. Cause of A549/DDP cells sensitivity to cisplatin may was due to maintaining cells in G0-G1 phases and ceasing them from entering S phase (62, 63) (fig.2). octamer-binding transcription factor 4 (OCT4) is a transcription factor
that functions in regulation of growth and metastasis of cancerous cells. As overexpressed levels of OCT4 in NSCLC has reported, X. Liu and colleagues showed that OCT4 mRNA and protein are in high levels in lung tumor tissues compared with adjacent normal tissues. Then, their study displayed that OCT4 was upregulated in A549/DDP cells than A549 sensitive cells. The results revealed that A549/DDP cells that were transfected with siRNA of OCT4 gene and treated with cisplatin displayed remarkable increased apoptosis in comparison to the control group (64). In other similar study, siRNA used against OCT4 increased gefitinib-induced apoptosis in PC9/GR cells compared with the matched group without siRNA, so OCT4 knockdown reversed gefitinib resistance (65). Heme Oxygenase-1 (HO1) which is known as an anti-oxidative and anti-apoptotic enzyme belongs to heat shock proteins family. Upregulation of this protein has been reported in multiple tumors. Hak-Ryul Kim et al. tested the hypothesis of HO1 inhibition and sensitizing of A549 cells to cisplatin. The results indicate that cisplatin treatment led to increased MAPK phosphorylation that in turn induced Nuclear factor (erythroid-derived 2)-like 2 (NRF2)-HO1 pathway and HO1 inhibition by siRNA in A549 cells led to cisplatin cytotoxicity augment, subG0-G1 fraction increasing and cell viability reduction in comparison with control cells without HO1 siRNA. Also, pro caspase-3 degradation was occurred only in HO-1 siRNA treated cells (66) (Fig.2). Forkhead box protein M1 (FOXM1) is an oncogenic transcription factor with a central function on cell cycle by promoting of G1/S transition and tumorigenicity progression. This transcription factor could form a complex with B-cat and TCF4/ Lymphoid enhancer-binding factor 1 (LEF1) to promote the WNT signaling pathway. Also, it is known to be a vital downstream target of Kirsten rat sarcoma viral oncogene (KRAS)/ Extracellular signal-regulated kinase (ERK) pathway. It is reported that upregulated FOXM1 is associated with chemo-resistance in several cancers. FoxM1 downregulation in cisplatin and docetaxel resistant lung cancer cell lines by siRNA could enhance the apoptotic effect of these chemotherapeutic agents through activation of c-Jun N terminal kinase (JNK) mitochondrial signaling pathway (67-70) (Fig.2). It is recently identified that cancerous inhibitor of PP2A (CIP2A) oncoprotein has a highlighted role in tumor progression. There are several studies that indicate CIP2A adjusting via RAS/ERK/ETS1 signaling pathway. Weil et al. investigated its role in cisplatin-resistant NSCLC by utilizing RNAi to knockdown CIP2A. Their results showed that silenced CIP2A increased apoptosis and chemo-sensitivity of cisplatin and impaired clonogenicity (71, 72)(Fig.2). Integrins are transmembrane heterodimer proteins
(mediating cell-ECM interaction) that have crucial functions in signal transduction events. In several cancers has been proposed that integrin β1 pathway by enhancing cell survival rate mediates Chemo-resistance. To investigate this hypothesis, Qin-Fang Deng et al. silenced integrin β1 in PC9/G cells by shRNA. The obtained results were in concordance with hypothesis which showed that integrin β1 knockdown elevated response to gefitinib. Also, other results indicated that this effect is due to reduced PI3K/AKT pathway activation and apoptosis induction by integrin β1 silencing (73) (Fig.2). Lately, long non-coding RNAs (lncRNAs) involvement in chemotherapy resistance has been spotted Bin Liu and et al. executed an experiment to find out the efficacy of AK001796 downregulation by siRNA on drug sensitivity of NSCLC cells. The gathered results displayed that AK001796 knockdown reduced cisplatin resistance and caused cell cycle arrest at G0/G1 phase and also enhanced number of apoptotic cells. Also, it was observed that Cell division protein kinase 5 (CDK5) and G2 and S phase-expressed protein 5 (GTSE5) cell cycle promoting factors were repressed though Cell cycle proteins cyclin C (CCNC) and BIRC5 apoptosis promoting factors were induced (74). L. Y. MA and et al in a record confirmed that TRPM2-AS lncRNA was upregulated in A549/DDP cells and subsequent downregulation of this RNA led to the induction of apoptosis and cell cycle arrest at subG0/G1 phase. Moreover, in the transfected cells with TRPM2-AS, siRNA p53-p66shc pathway was upregulated. Therefore, it could be concluded that the lowered TRPM2-AS expression by siRNA through activating of p53-p66shc pathway resensitizes cells to cisplatin (45).

EMT is a reversible process that results in loss of epithelial characteristics and gaining mesenchymal traits. It has been shown that EMT-promoting transcription factors or proteins, including SNAIL, SLUG, Twist-related protein 1 (TWIST1), Zinc finger E-box-binding homeobox 1 (ZEB1), Zinc finger E-box-binding homeobox 2 (ZEB2) and Matrix metalloproteinases (MMPs) through guidance of diverse signaling pathways, such as RAS/RAF/MEK/ERK, JNK, PI3K/AKT and WNT/B-catenin are regulated. It is also reported that EMT is related to drug resistance of NSCLC (75-77) (Fig.2). The results of different reports indicated that down regulation of crucial genes that contribute EMT, revive sensitivity of cells via apoptosis induction. One of these genes is TWIST1, according to observation of TWIST1 upregulation in A549 cells. Wen-Lei Zhuo and colleagues showed that TWIST1 downregulation could sensitize cells to chemotherapeutic agents. As expected TWIST1-siRNA treated cells
showed enhancement of apoptosis induction by cisplatin. Due to the enhanced expression of P-JNK in A549-siTWIST cells containing DDP, it is possible to consider that TWIST1 knockdown could increase cisplatin sensitivity of A549 NSCLC per MAPK/mitochondrial pathway (78). Also, it was reported that TWIST1 knockdown by siRNA concurrently sensitized H1299 NSCLC cells to arsenic trioxide (ATO) and elevated PARP cleavage. Knockdown of TWIST1 through regulating mitochondria fragmentation and reactive oxygen species (ROS) generation mediates this increased ATO-sensitization (79). Based on this observation, TWIST1/EMT signaling pathway was activated in A549-CD74-ROS1 G2032R mutated cells, which were resistant to crizotinib. Wenfeng Gou And et al also silenced TWIST1 gene with specific siRNA and their results showed that TWIST1 downregulation in combination with crizotinib could remarkably improve sensitivity of the cells to crizotinib compared with crizotinib treated group lonely (80). Koichi Azuma and colleagues carried out a study and their results suggested that switch from EGFR family to fibroblast growth factor / fibroblast growth factor receptor (FGF/FGFR) family signaling pathway is a cause of afatinib resistance acquisition. FGFR1 activation also leads to the activation of AKT and ERK pathways and subsequently promotes cell survival/growth and malignant transformation of cancerous cells. It was exhibited that EMT-transcription factors (EMT-TFs), such as SNAIL and TWIST1 in afatinib resistant cells was elevated. since TWIST1 downregulation leads to FGFR1 decrease, in this study TWIST1 knockdown by specific siRNA blocked AKT phosphorylation and overcame drug resistance to afatinib (81) (Fig.2). To further understand the exact role of TWIST1 silencing in cisplatin increased sensitivity can point out to an experiment carried out with H-O Jin and et al. In this study it was indicated that MCL1 (a BCL2 family member) is lowered after mTOR/ Ribosomal protein S6 kinase beta-1 (S6K1) reduction in A549 and H1299 cells. M-TOR inhibition occurred after 5' AMP-activated protein kinase (AMPK) activation which in turn was a result of TWIST1 knockdown by siRNA. Therefore, it could be concluded that TWIST1-siRNA could trigger AMPK induced mTOR/S6K1 inhibition causing MCL1 decrement. Finally, MCL1 decrease following TWIST1 knockdown enhanced PARP cleavage and apoptosis progression mediated by cisplatin (82) (Fig.2). Another gene is SNAIL1, an EMT-transcription factor, increased levels of SNAIL in A549 cells was observed by Wenlei Zhuoa and et al, while they came up with this thought that cause of cisplatin resistance may be due to SNAIL1 overexpression. Data gained from this study indicated that solely silencing of SNAIL rarely induced apoptosis of cells, but
when SNAIL depletion occurs with cisplatin usage simultaneously, A549 cells was sensitized to apoptosis mediated by cisplatin. This enhanced chemosensitivity was co-existed with JNK/mitochondrial pathway activation (83).

Nevertheless, EMT process has exhibited significant roles in promoting of chemotherapy resistance in various cancers such as lung cancer. In a study carried, it was demonstrated that the expression of EMT-related transcription factors, such as SNAIL, SLUG, TWIST, ZEB1, P120 catenin and Protein arginine N-methyltransferase 1 (PRMT1) were enhanced in NSCLC cells. PRMT1 methylates TWIST1. p120 catenin via binding to kaiso factor puts down kaiso transcriptional repression effect on WNT/B-catenin path. So, WNT/B-catenin resumes its function and by activating EMT-transcription factors triggers EMT process. Hence, they put up these two genes to knockdown and their results displayed that siRNA usage against PRMT1 and P120 catenin reversed EMT to overcome TKIs resistance and elevated erlotinib sensitivity (84, 85) (Fig.2). EGFR overexpression is correlated with the progression of various cancers with the inclusion of lung cancer. There have been efforts to hinder EGFR expression in NSCLC. For example Min Zhang et al. tested RNAi approach; data obtained from their experiment showed that EGFR gene targeting by sequence-specific siRNA on A549 and SPCA1 resulted in increased sensitivity of A549 cells four-fold and SPCA1 cells seven-fold to cisplatin, also number of the cells and colonies displayed reduction and attenuated migration and invasiveness compared to the control cells (86) (Fig.2). IGF1R plays a significant role in acquired secondary drug resistance to EGFR-TKIs by activating of downstream pathway of EGFR signaling path. Also, IGF1R is engaged in EMT process. Zhou and et al evaluated IGF1R role-playing in acquisition of resistance to EGFR-TKIs through EMT process on PC9/GR and H460/ER gefitinib and erlotinib-resistant lung cancer cell lines, respectively. The also applied IGF1R siRNA and demonstrated that the sensitivity of the cells to these drugs is restored and EMT related transcriptional and morphological features were returned (57) (Fig.2). Since β-catenin accumulation in the nucleus results in EMT-TF, such as ZEB1, TWIST1, SNAIL and SLUG activation and later cell transformation, Ichwaku Rastogi and et al in their experiment used mir200a to target ZEB1 and specific siRNA to target β-catenin in H1270 erlotinib resistant/ SU11274resistant (ER/SR) cells following by erlotinib or SU11274 treatment. Data obtained from this study confirm that the down regulation of ZEB1 or β-catenin by suppressing of EMT process leads to increase of erlotinib/ SU11274 sensitivity. As the conclusion point can say that
inhibition of key signaling pathways resulting in EMT such as β-catenin path could be impressive in overcoming EMT-mediated TKI-resistance in NSCLC (87, 88) (fig.2). In another study, when ZEB1 was down-regulated by siRNA in HCC4006ER cells, it reverted erlotinib sensitivity to the cells to the same IC$_{50}$ value of HCC4006 cells that were not resistant (89). OCT4 increased expression was also reported by Yu-Chih Chen and et al in LC-CD133$^+$ cells; this transcription factor elevation was accompanied with refractory traits of chemotherapy. It was previously reported that siRNA treatment against OCT4 led to the reduction of invasion and colony formation abilities and improvement of chemotherapy compared with the control group (90). Thymidylate synthase (TS) is one of key enzymes, which are hindered by pemetrexed. TS inhibition leads to declined DNA synthesis through decreased Deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) turnover. The production of thymidine is required for circumvent cellular death which caused by pemetrexed. TS gene upregulation and subsequently Pemetrexed resistance in SCLC has been reported. L-Y Chiu and et al. downregulated TS by shRNA-expressing lentivirus in CL1/A200 and CL1/A100 cell lines. It was reported that TS inhibition enhanced Pemetrexed sensitivity and also caused proliferation, migration and invasion reduction (91). AMPK-related protein kinase 5 (ARK5) is a member of AMPK family that is directly activated by AKT. It is also known as a mediator of AKT pathway, causing cell proliferation, survival, invasion and metastasis. Also, it has been remarked that ARK5 overexpression is associated with MMPs (MMP2/9), key enzymes of metastasis, activation. Minghui Li and et al showed that ARK5 might impress cisplatin sensitivity of NSCLC using siRNA silenced this gene. Coming out from the results showed that siRNA treatment augmented cisplatin sensitivity of NCI-H1229 cells compared to the control cells (92, 93) (fig.2).

Plus two main processes (apoptosis and EMT) and consisting proteins in the chemotherapy resistance, there are other pathways and proteins that their misexpression could result in drug resistance and hindrance of their role playing could reverse this effect. One of the conserved signaling pathways of evolution is NOTCH1 signaling path, which functions on apoptosis, proliferation and differentiation through regulating of EMT-transcription factors, such as SNAIL, SLUG , TWIST1 , c-MYC or BCL2 after NICD (notch intracellular domain) release. Recently researchers have linked this gene with drug sensitivity of various tumors. F. Deng and colleagues reported that after NOTCH1 gene silencing by siRNA chemosensitivity of A549 cells to cisplatin
was dramatically improved (94-97) (Fig.2). C-X-C chemokine receptor type 4 (CXCR4) is a G-protein that is coupled with transmembrane receptor, which after activation by its ligand plays significant roles in cell proliferation, cell survival and metastasis via activating of PI3K/AKT, MAPK/ERK and WNT/B-catenin canonical signaling pathways. CXCR4 is overexpressed in cisplatin resistant lung cancer patients than the patients that are sensitive to this drug; also, CXCR4 mediates cisplatin resistance in NSCLC in a Cytochrome P450 1B1 (CYP1B1)-dependant way. CYP1B1 is a member of cytochrome P450 enzymes. This group of enzymes is involved in metabolism of various cytotoxic drugs. It is referred that CYP1B1 reduces cytotoxic effect of drugs. Different studies revealed that CXCR4 inhibition could reverse cisplatin resistance and decrease tumor cell proliferation (98-100) (Fig.2). Hypoxia means reduced oxygen levels of tissues for any reason. In hypoxic condition more than 40 genes expressions are induced by Hypoxia-inducible factor 1 (HIF1). These activated genes could play essential roles in angiogenesis, survival, growth, metastasis and apoptosis. Also, hypoxia contributes to metabolic and genetic changes of cancerous cells like resisting them to chemotherapeutical agents. Exposure of NSCLC cells to 0.5% O₂ increased resistance to cisplatin and doxorubicin in contrast to the cells were exposed to normal concentration of O₂ (19%). To verify HIF1α involvement in drug resistance of lung cancer, Xianrang Song et al. downregulated this gene by targeting of it with a lentivirus vector expressing HIF1α shRNA. They confirmed that hypoxia may lead to the HIF1α accumulation and its regulation of gene transcription subsequently. Finally, hypoxia-induced chemoresistance on A549 and SPCA1 to cisplatin and DOX were reversed (101) (Fig.2). In a HIF1A dependent or independent way PI3K/AKT leads to VEGF overexpression. VEGF is a crucial angiogenesis inducer, which by activating of VEGFR initiates different signaling pathways, leading to proliferation, migration and survival. Also, PI3K/AKT/mTOR signaling pathway has vital functions on proliferation, angiogenesis, survival and motility. Regarding to this fact, the treatment against angiogenesis are promising for lung cancer. Laura Espana-Serrano1 and Mahavir B Chougule in an experiment used PF-04691502 and VEGF siRNA to inhibit VEGF. PF-04691502 which is dual inhibitor for MTOR and PI3K in a synergistic interaction with VEGF siRNA, showed a significant long-term proliferation, migration, colony formation and cell viability decrease in A549 and H460 cells compared to the single drug treatment. This combination treatment also displayed angiogenesis reduction and enhanced anticancer activity of PF-04691502 (37) (Fig.2). (Hepatocyte growth factor (HGF)/
hepatocyte growth factor receptor (HGFR) or MET interaction and activating downstream intracellular signaling pathways like ERK/MAPK or PI3K/AKT is a potential key contributor to EGFR-TKIs or cetuximab resistance. Seiji Yano et al. showed that MET targeting by specific siRNA could eliminate HGF-induced gefitinib resistance. In addition, it was shown that AKT and ERK1/2 phosphorylation restored by HGF in PC9 or HCC827 cell lines. They also showed that HGF/MET axis induced gefitinib resistance by activating anaplastic lymphoma kinase (ALK) pathway. In another study, MET was downregulated by siRNA that led to cetuximab resistance abolishment in LXFA529L and LXFA1647L cell lines (102-104) (fig.2). Yes-associated protein 1 (YAP1) as a transcription coactivator is a direct downstream of JNK pathway that after localization to nucleus in association with TEAD promotes gene expression like BIRC5 and ABCGA2 by binding to their promoters. ABCG2 is a chemotherapy resistance contributor in lung cancer. It was observed that YAP1 down-regulation by siRNA in A549 and H460 cells led to ABCG2 decreased expression and enhanced doxorubicin sensitivity of the cells (105-107)(Fig.2). Significant role of P38-MAPK- Heat shock protein 27 (HSP27) axis in CSC mediated cisplatin-resistant lung cancer has been proved by targeting of HSP27 with siRNA improved prognosis and led to decreased cisplatin resistance (15) (Fig.2).

2-2- Combination of dual or more gene silencing in chemotherapy

Targeting genes that resulting in chemo-resistance from diverse pathways could have better results. By attacking more than one gene, could be hopeful that in a subset of tumors with more than one singular gene involved in drug resistance, tumors will again be sensitive to cytotoxic effects of drugs (108). In another study, HE-GUO JIANG and colleagues tried to figure out two distinct genes, ATR and REV3 down-regulation in cisplatin sensitivity of A549, A549/cip, and sk-mes-1 cells. POL ζ, a translesion synthesis (TLS) polymerase consists of a catalytic subunit REV3, which plays important roles in DNA damage response (DDR) and DNA damage tolerance (DDT). ATR is another enzyme that is involved in DNA repair by phosphorylation of downstream gene Checkpoint kinase 1 (CHK1). Either of these genes deficiency sensitizes cells to cisplatin. According to this idea, research consequence indicates that REV3 and ATR co-depletion in A549/CIP and sk-mes-1 cells in comparison with REV3 or ATR individual depletion enhances cisplatin cytotoxicity (109)(Fig.1). In another similar study regarding to this fact that enhanced DNA damage repair is the main cisplatin resistance mechanism. Co-depletion
of POLQ from TLS/Base Excision Repair (BER) repair pathway plus BRCA2 or FANCD2 component of HR/FA pathway was tested. Co-silenced polQ and BRCA2 or FANCD2 reduced resistance to cisplatin, which was accompanied by impairment of double strand breakage (DSB) repair compared to the individual depletion of BRCA2, FANCD2, or POLQ. The colony formation assay gave the same result that in cells co-treated with polQ and BRCA2 or FANCD2 siRNA, number of colonies were decreased compared to cells treated with individual siRNA of these genes (110, 111) (Fig.1).

2-3- simultaneous drug application and gene silencing

Recently different studies have focused on simultaneous delivery of siRNA targeting resistance genes and chemotherapeutical agents. To achieve this purpose, there is a need for a vehicle for dual carrying of siRNA and drugs and releasing them at the same time. Nanoparticles are the best choice for following this aim.

Quantum dots (QD) nanoparticles used by Jinming Li et al. to deliver siRNA plus different anticancer drugs (carboplatin, paclitaxel and doxorubicin) simultaneously on A549 lung cancer cells. SiRNA was used in this study to target BCL2 gene that is an important antiapoptotic gene. This co-delivery led to induction of G2/M phase arrest in larger cell population and elevation of apoptosis rate compared to the free drug-treated cells. Also, carboplatin, paclitaxel, and doxorubicin combination with BCL2 siRNA led to three to four-fold increased cytotoxicity of these drugs in A549 cells compared to free drug treatment (33) (Fig.1). Survivin (BIRC5) as a member of IAP family that collaborates with XIAP and interference with caspases -3/-9. It was found to be highly expressed in multiple malignant tumors, particularly in drug-resistant cancerous cells. Mixture of survivin siRNA and cisplatin in A549/DDP cells were employed. To increase siRNA delivery efficacy polyglutamate derivative polymer brush had been used. Results indicated that cytotoxic and apoptosis-inducing effect of DDP combined with PPGS/si-survivin was enhanced. also IC50 of DDP was reduced in cells treated with DDP and pretreated with PPGS/si-survivin polyplex compared to DDP usage lonely. Finally, PPGS/si-survivin polyplex, showed a synergistic effect when combined with cisplatin on killing cancer cells of lung adenocarcinoma (40, 112) (Fig.1). BCL2 and surviving are well-known apoptosis delaying proteins that are down regulated with specific siRNAs, and in combination with cisplatin in HA-Based nanoparticles. The results showed that simultaneous BCL2 and survivin silencing plus
cisplatin led to a reversal of resistance (108) (Fig.1). Signal transducer and activator of transcription 3 (STAT3) is a regulator of gene that is involved in angiogenesis (HIF-1α, VEGF), cell cycle regulation (c-Myc) and apoptosis inhibition (Bcl-2, survivin). The overexpression of STAT3 could contribute to chemo-resistance. Wen-Pin Su et al. demonstrated PLGA nanoparticles loaded with paclitaxel and STAT3 siRNA could reduce cellular resistance to paclitaxel in both resistant (A549/T12) and un-resistant (A549) lung cancer cells (113, 114) (fig.2). There are studies hypothesizing that suppression of pump and non-pump resistance in combination with chemotherapeutic agents simultaneously could show more potency and efficacy in overcoming drug resistance. Multidrug resistance-associated protein 1 (MRP1), regulated by PI3K/AKT/NRF2 path, is a P-glycoprotein efflux pump that belongs to superfamily of ATP-binding cassette transporters. It is able to emit various molecules from cell membrane such as anticancer drugs. As previously mentioned, BCL2 is an antiapoptotic protein, which inhibits cytochrome-c release from mitochondria. A constructed multifunctional nanocarrier-based delivery system (NDS) to co-deliver DOX along with MRP1 siRNA and BCL2 siRNA to target pump and non-pump resistance in Human MDR H69AR lung cancer cells was applied. The obtained results showed efficient apoptosis induction and annihilation of drug-resistant cells. Also, it was indicated that simultaneous usage of DOX and siRNAs increased chemotherapy effectiveness and efficacy to a level that cannot be reached by other treatment groups, including free DOX, liposomal DOX, and liposomal DOX with only one type of siRNA or mixture of both liposomal siRNAs without DOX (115, 116) (Fig.2).

It is possible that one protein via two distinct pathways could result in drug resistance. IGF1R is a member of the tyrosine kinase family (RTK) family that consists of two A and two B subunits on transmembrane of cells. IGF1R in association with different adaptor proteins triggers several downstream pathways that eventually leads to apoptosis resistance, angiogenesis, tumor cell growth, and invasion. Results gained from several studies indicate a significant role of IGF1R in EGFR-TKI resistance. Shali H et al. investigated the effect of dual delivery of IGF1R siRNA and DOX by chitosan nanoparticles on the viability, migration and apoptosis of A549 cells. Their results revealed that ChiNP/siRNA/DOX/CMD decreased cell viability, increased drug cytotoxicity, and induced apoptosis, inhibited migration compared with the treatment of free DOX, ChiNP / DOX or other control groups. In a part of the study, it was showed that IGF1R and DOX co-delivery could significantly down-regulate MMP1, VEGF and STAT3 expression.
downstream genes of IGF1R, which are involved in metastasis and angiogenesis, in comparison with the free DOX or other groups (34) (Fig.2). HMGA2 belongs to high mobility group AT-hook proteins that by changing chromatin structure and interaction with various transcription factors plays a significant role in several biological processes, such as differentiation, EMT, apoptosis, invasion and cell growth (117, 118). Encapsulated DOX and HMGA2 siRNA in chitosan nanoparticles and its application on A549 cells demonstrated the more efficient inducing cytotoxicity on A549 cells and potential in the induction of apoptosis and effective in inhibiting migration compared with the individual treatment (32).

3- *Invivo* experiments displaying chemotherapy drug efficacy enhancement after RNAi application

Due to hopeful results gained from *in vitro* studies, there has been a rise in *invivo* researches using lung cancer-bearing mice to examine chemotherapy and RNAi combination, which are reported anticipant results.

Co-delivered DOX and BCL2 siRNA in H1299 cells by nanoparticles induced apoptosis of the cells more effectively and efficiently than DOX or BCL2 siRNA alone. The effect of simultaneous intravenous injection of BCL2 siRNA and DOX on H1299 tumor-bearing mice was also evaluated. and combination of BCL2 siRNA and DOX more effectively induced apoptosis, inhibited lung cancer growth and reduced tumor volume significantly than individuals (119) (Fig.1). Dual delivery of siRNA targeting survivin plus cisplatin was examined on NSCLC cells *in vivo* and *in vitro* by George Mattheolabakis et al. It was reported that survivin siRNA and cisplatin combinatorial treatment sensitized A549/DDP cells to cisplatin and had the same effects on tumor-bearing mice. It led to 65% tumor growth inhibition versus 4% inhibition by cisplatin usage lonely (120) (Fig.1). Hongwei Tian and colleagues constructed a plasmid coding shRNA against survivin gene to inhibit this gene expression and used in combination with cisplatin *in vivo* and *in vitro*. *In vitro* results indicated that pshSUR+cip significantly decreased proliferation, the IC_{50} value of cisplatin and increased apoptosis compared with other groups like cisplatin lonely. *In vivo* results showed that combinational-therapy led to the enhancement of antitumor effect in comparison with pshSUR or cisplatin usage alone (35) (Fig.1). Also, Oleh Taratula and colleagues constructed a MSN-based drug delivery system to deliver dox, cisplatin and two siRNAs targeting BCL2 and MRP1 simultaneously by inhalation to mice lungs. MRP1 is
responsible gene for pump, while BCL2 is responsible for non-pump resistance. Simultaneous suppression of both resistance types is capable to increase the efficacy of anticancer drugs. Such dual delivery of two siRNAs with chemotherapeutical agents could have better consequences, as results showed that suppression of pump and non-pump resistance by specific siRNA raised drug cytotoxicity (121) (fig.2). Lung adenocarcinoma cell lines from mice were picked to examine REV3 down-regulation on cisplatin response. Cells were transfected with retrovirally expressed shRNAs designed to target REV3. REV3L shRNAs sensitized cells to cisplatin significantly relative to vector control-infected group. To examine REV3L depletion in vivo, REV3L knock downed and control group were transplanted to mice. REV3L deficient transplants demonstrated an increased response to cisplatin compared to the control group (122). Mei-Hua Qu and colleagues checked out docetaxole (DTXs) and BCL2 siRNA incorporated role in lung cancer model with the purpose of overcoming MDR and increasing of the efficacy of chemotherapeutic agents. PEGYlated liposome consists of siRNA specific to BCL2 and DTX delivered to A549 and H226 cells. Attained data exhibited that lipoDTX siRAN significantly reduced cell proliferation, reduced the IC50 value of DTX, increased subG0/G1 phase, induced apoptosis and superior caspase activity compared to lipoDTX or free DTX. As expected lipo-DTX-siRNA showed remarkable tumor growth inhibition on xenograft tumor mouse model compared with free DTX treatment (30) (Fig.1). EPHA2 receptor tyrosine kinase is a member of ephtransmembrane tyrosine kinases family. The EPHA2 expression is associated with angiogenesis, metastasis and tumor growth. Improved cellular sensitivity to lipoplatin (liposomal cisplatin) on cell proliferation, tumor sphere growth, and cell migration by knocking down of EPHA2 gene with siRNA in lung cancer cell lines and mouse lung carcinoma was reported by Hung-Yen Lee and et al (123). As previously mentioned, cisplatin-made adducts by cessation cell division and activating apoptosis pathway destroys cancerous cells. The functional protains in cell cycle check-points monitor DNA damages to repair. It makes an arrest in G2/M phase or mitotic phase. If the cell could not be repaired, cell death would occur. Mitotic arrest deficient (MAD) is a checkpoint protein that plays an essential role in the correct segregation of chromosomes during mitosis. The opposite effect can be considered as a treatment alteration. Mad2 silencing leads to mitosis exist and enhanced levels of segregate errors and eventually cell death. Ana Vanessa Nascimento et al. managed a study on two groups of mice bearing subcutaneous cisplatin resistant and sensitive lung adenocarcinoma to assess the efficacy of
Mad2-siRNA and CTX combination on tumor growth inhibition. This co-therapy contributed to tumor inhibition improvement, especially in cisplatin-resistant tumors and decreased cisplatin effective dosage, resulting in a minimum toxic effect (39). The results of another study has announced that nanoparticle-based miRNA 34a and KRAS siRNA delivery plus cisplatin-mediated chemotherapy in a genetically engineered lung cancer mouse model prolonged survival in comparison with the individual treatment of chemotherapy (124) (Fig.2).

4-Conclusion

Among cancers, lung cancer with the most morbidity and mortality rate remains the fatalist one. A reason for this challenge is chemotherapy or targeted therapy resistance of cancerous lung cells. Lung cancer accounts for 25% of cancer-related death in the worldwide that makes it the most frequent and deadliest cancer. In diverse treatments of lung cancer, such as surgical resection, radiotherapy, targeted therapy, immunotherapy and chemotherapy, the 5-year survival rate is less than 20%. Metastasis and acquired resistance to existing remedies could be considered as the reason of this phenomenon. To overcome drug resistance in lung cancer, new method explorations are necessary. A potential strategy is downregulating resistance causing genes (15, 73, 108, 125, 126). RNAi when is combined with traditional chemotherapy could be a perfect choice because of more efficacy, safety and potency and less expense and side effects (34-38). According to the received reports from multiple in vitro and in vivo studies the combined approach is getting more and more achievements. By silencing genes resulting in drug resistance from distinct pathways, the cytotoxic effect of drug raises and number of cancerous cells ruining enhances because of reversed resistance and resensitized cells. Although co-treatment with siRNA and chemotherapy agents accompanies mentioned advantages, there are still drawbacks. Poor stability of siRNA in the biological milieu and transient effect because of short half-life and quick degradation due to serum nucleases or renal clearance and Immune system induction are some of these drabacks. Even after reaching the cell anionic charge and hydrophilic nature of siRNA impedes entering siRNA into cells. In cytoplasm, there are other obstacles, if siRNA is entered by endocytosis, RNAase enzymes encircled in lysosome will degrade them. The second problem is about dividing cells that siRNA concentration gets diluted in every division. Off-target effect that originates from relative complementation of siRNA and
sequence of other than target mRNA contributes to their silencing is another one. Out of obstacles mentioned can draw a conclusion that a delivery system for siRNA and CTXs should be protective against the circulatory environment and be efficient enough to deliver loadings of the drugs to the exact site of action in cancerous tissues; consequently, leads to the maximum accumulation in there. Also, it should be safe and not be a stimulator of immune system. In addition, it must help loads entering into cells and escaping lysosomal degradation (36, 127-130). According to what had been discussed, it can be drawn a conclusion that after DNA damage occurrence (naturally or by chemotherapeutic agents), two distinct pathways lie ahead of cell fate, DNA damage repair or apoptosis (131) (Fig.1). As mentioned, drug resistance may occur through various pathways that end up in EMT-inducing or DNA damage repairing proteins enhancement as well as apoptosis-promoting proteins reduction. About genes and pathways pointed out in this manuscript can explain that after activation of cell membrane receptors such as IGFR, EGFR, FGFR, VEGFR, CXCR4, MET, INTEGRINS, NOTCH1 and FRIZZLED via their ligands, downstream intracellular pathways containing JAK/STAT, RAS/RAF/MEK, PI3K/AKT, NICD, B-catenin/TCF/LEF stimulation occurs. These pathways activation eventually contribute to regulation of genes involved in drug resistance. (97, 99, 100, 132-134) (fig.2). By downregulating of which through RNAi approach in association with chemotherapy agents, hope of overcoming resistance against chemo and targeted therapy gets increased.

References


Figure 1. Schematic diagram of two distinct pathways activation after DNA damage. Subsequent to DNA damage occurrence cells either choose apoptosis path by activating of P53 and downstream agents that lead to cell death or opt DNA repair pathway and via different DNA repair processes repairs the damage.

Figure 2. Schematic diagram of activation of drug resistance promoting genes mentioned in this manuscript, intracellular signaling pathways (JAK/STAT, RAS/RAF/MEK, PI3K/AKT, NICD, B-catenin/TCF/LEF) activation after receptors (IGFR, EGFR, FGFR, VEGFR, CXCR4, MET, INTEGRINS, NOTCH1 and FRIZZLED) stimulation contributes to upregulation of genes involved in EMT promotion or apoptosis inhibition.
Abbreviations

AMP-activated protein kinase (AMPK), AMPK-related protein kinase 5 (Ark5), Anaplastic lymphoma kinase (ALK), Apoptotic protease activating factor 1 (APAF1), Argonaute (AGO), Arsenic trioxide (ATO), ATP-binding cassette (ABC), Base Excision Repair (BER), B-cell lymphoma 2 (BCL2), B-cell lymphoma-extra-large (BCL-XL), Bcl-2 homologous antagonist/killer (BAK), BCL2-associated X protein (BAX), Cancer stem cells (CSCs), Cancerous inhibitor of PP2A (CIP2A), Casein kinase 2 (CK2), Cell cycle proteins cyclin C (CCNC), Cell division protein kinase 5 (CDK5), Checkpoint kinase 1 (CHK1), c-Jun N terminal kinase (JNK), Computed tomography scan (CT scan), C-X-C chemokine receptor type 4 (CXCR4), Cytochrome P450 1B1 (CYP1B1), Deoxycytidine monophosphate (dTMP), Deoxyuridine monophosphate (dUMP), Diallyl disulfide (DADS), DNA damage response (DDR), Docetaxole (DTXs), Double strand breakage (DSB), Doxorubicin (Dox), EMT-transcription factors (EMT-TFs), Enhancer of zeste homolog 2 (EZH2), Epidermal growth factor receptor (EGFR), Epithelial–mesenchymal transition (EMT), Erlotinib resistant/ SU11274resistant (ER/SR), Excision Repair Cross-Complementation Group 1 (ERCC1), Extracellular signal–regulated kinase (ERK), Fanconi anemia / breast cancer susceptibility protein (FA/BRCA), Fanconi Anemia Complementation Group L (FANCL), Fanconi anemia group F protein (FANCF), Fibroblast growth factor (FGF), Fibroblast growth factor receptor (FGFR), Forkhead box protein M1 (FOXM1), G2 and S phase-expressed protein 5 (GTSE5), Heat shock protein 27 (HSP27), Hepatocyte growth factor (HGF), Hepatocyte growth factor receptor (HGFRI), Hypoxia-inducible factor 1 (HIF1), Insulin-like growth factor 1 receptor (IGF1R), Interleukins (ILs), Kirsten rat sarcoma viral oncogene (KRAS), Long non-coding RNAs (lncRNAs), Lymphoid enhancer-binding factor 1 (LEF1), Matrix metalloproteinases (MMPs), Mediator complex subunit 19 (MED19), MicroRNA (miRNAs), Mitogen-activated protein kinase (MAPK), Mitotic arrest deficient (MAD), Multidrug resistance-associated protein 1 (MRP1), Multiple drug resistance (MDR), NA damage tolerance (DDT), Nanocarrier-based delivery system (NDS), Non-small cell lung cancer (NSCLC), Nuclear factor (erythroid-derived 2), -like 2 (NRF2), Nucleotide excision repair (NER), Octamer-binding transcription factor 4 (OCT4), Phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K), Promyelocytic leukemia protein (PML), Protein arginine N-methyltransferase 1 (PRMT1), PTGS (post transcriptional gene silencing), Pyruvate kinase isozymes M2 (PKM2), Quantum dots (QD), Reactive oxygen species (ROS), RNA interference (RNAi), RNA-induced silencing complex (RISC), S6 kinase beta-1 (S6K1), Short hairpin RNAs (shRNAs), Signal transducer and activator of transcription 3 (STAT3), Small cell lung cancer (SCLC), Small interfering RNA (siRNA), Thymidylate synthase (TS), Topoisomerase 2-binding protein 1 (TOPBP1), Transcription factor 4 (TCF4), Translesion synthesis (TLS), Twist-related protein 1 (TWIST1), Tyrosine kinase family (RTK), Tyrosine kinase inhibitors (TKIs), Vascular endothelial growth factor (VEGF), Wingless/Integrated (WNT), Yes-associated protein 1 (YAP1), Zinc finger E-box-binding homeobox 1 (ZEB1), Zinc finger E-box-binding homeobox 2 (ZEB2).
Highlights

*Invitro* experiments displaying chemotherapy drug efficacy enhancement after RNAi application

Drug application after gene silencing

Combination of dual or more gene silencing in chemotherapy

Simultaneous drug application and gene silencing

*Invivo* experiments displaying chemotherapy drug efficacy enhancement after RNAi application
Figure 1
Figure 2