Lung cancer drug resistance and DNA damage signaling

Peilin Zhang
Putnam General Hospital Laboratory, Hurricane, WV 25526, USA
Tel.: +1 304 757 1770
Fax: +1 304 757 1736
Peilin.Zhang@hcahealthcare.com

The most challenging problem in cancer therapy is tumor resistance to treatment modalities, such as radiation and chemotherapy. There exist two types of resistance to DNA damage-inducing agents: first, naturally occurring resistance and, second, therapy-induced resistance. Advances in understanding the molecular mechanism that regulates the DNA damage-signaling pathway makes it possible to better define the key regulatory targets involving the development of tumor resistance. In this brief report, we summarize the recent advances in DNA damage signaling, and discuss the potential targets for drug development and better design of clinical trials for cancer patients, especially those with lung cancer.

Keywords: chemotherapy, DNA damage, lung cancer, radiation therapy

Traditional cancer therapy consists of surgery, chemotherapy and radiation therapy. For many solid tumors, regardless of their cell origins, surgical resection is the optimal modality for cure. Too often, cancer presents at advanced stages, surgery becomes palliative and adjuvant therapy, such as radiation or chemotherapy, is the only means for a patients survival. For any advanced malignant tumors (clinical Stage III and IV), some type of radiation or chemotherapy is needed. Some cancer types, such as small cell lung cancer (SCLC) and seminomatous germ-cell tumor of the testis, are extremely sensitive to cytotoxic radiation and/or chemotherapy, while others, such as sarcomas of mesenchymal origin, glioblastoma, or non-small cell lung cancer (NSCLC), are much less sensitive to the same therapeutic agents. The question as to why different tissues in the body react so differently to the same cytotoxic agents has long been puzzling clinical oncologists. Obviously this question is directed to the initial response of the tumor to the cytotoxic agents, namely, the 'natural resistance' of the tumor. Equally, if not more important, is the fact that the tumor is initially responsive to treatment for a short period of time, it become highly resistant to the same therapeutic agents. How the tumor cells develop resistance after the initial response to the tumor-killing agents remains a challenge to the medical community. In the discussion below, we report on the recent advances of molecular oncology in the context of both naturally occurring and acquired tumor resistance.

Natural versus acquired tumor resistance

Theoretically speaking, any tumor can be killed by cytotoxic agents if the maximal dose is used. Within the human body, the tumor is surrounded by normal tissues and cytotoxic agents cannot be administered without limit. The goal of cancer treatment is to maximize tumor cell killing whilst minimizing normal tissue damage. Using standard chemo and radiation dosage, some tumors respond remarkably well and the tumor disappears after treatment, while others do not respond at all. The term 'natural resistance' refers to the tumors that do not respond, or are only minimally responsive to cytotoxic agents. There are a few tumors that are exceptionally sensitive to treatments including SCLC, seminomatous germ-cell tumor and some of the lymphomas and leukemias. There are also a few highly resistant tumors, and most of these are of mesenchymal origin, such as sarcomas of various kinds, glioblastomas and others such as NSCLC. Many clinical tumors fall in between in regard to sensitivity/resistance to therapy – tumor response is partial and after a short time, the tumor becomes completely resistant (therapy-induced resistance, acquired). Therapy-induced resistance appears more common and more challenging. The underlying mechanism of acquired resistance, for 10 years, was thought to be related to the multi-drug-resistance gene (*MDR*) [1]. When the DNA-damage signaling pathway was discovered, the many signaling molecules were found to be critical for DNA damage sensitization through genetic and biochemical analyses. These molecules appear to a play a critical role in DNA damage signaling and cancer sensitivity to DNA damage-inducing therapies, such as radiation and chemotherapy.

DNA damage signaling pathway & cancer resistance

In response to DNA-damaging agents, such as ionizing radiation (IR), ultraviolet (UV) radiation and chemical compounds, a cellular mechanism has been developed to protect the genetic

integrity and prevent DNA from mutations and carcinogenesis. This DNA damage-signaling pathway is initiated and centrally regulated by *ATM kinase*, a gene mutated in the rare genetic disease ataxia telangiectasia (AT) [2]. Activated *ATM kinase* leads to three major cellular events:

- Cell-cycle arrest
- DNA damage repair
- Apoptosis when DNA damage becomes irreparable

In a mouse model, *ATM*-knockout mice recapitulate most of the clinical manifestations of AT patients, characterized by [3,4]:

- Multiple system defects
- Predisposition to a variety of cancers
- Extremely sensitive to DNA-damaging agents

The *ATM* mutant cells, similar to the fibroblasts or lymphocytes of AT patients, are very sensitive to agents that cause double-strand DNA breaks, such as IR, indicating the critical role of ATM kinase in the DNA damage-signaling pathway. The *ATM*-related protein, ATR, shows many similarities in *in vitro* biochemical studies to *ATM* in response to DNA damage, but the fundamental difference is that the ATR knockout mice showed embryonic lethality and chromosome breakage that are significantly different from those in the *ATM*-null mice and the AT patients [5].

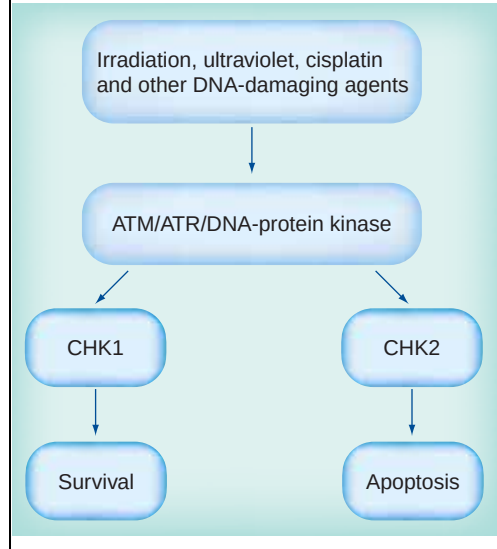
Once ATM kinase is activated in response to DNA damage signals, many direct downstream ATM targets are activated through phosphorylation. These targets include the nuclear c-Abl tyrosine kinase [6], the p53 tumor-suppresser protein [7–9], the checkpoint kinase (CHK)2 serine/threonine kinase [10], and the p34 subunit of replication protein A (RPA) [11], CHK1 [12,13], MDM2 [14], BRCA1 [15,16], NBS/p95 [17], FANCD2 [18] and SMC1 [19]. There are many new ATM targets that have recently been identified, and these targets all play important roles in cell-cycle regulation, DNA damage repair and apoptosis in cell-culture studies individually [20]. There are many overlapping features in response to DNA damage in mice with targeted deletion of individual genes described above. How important the interactions are between these important molecules is gradually emerging at the physiologic level.

Two of these direct *ATM* targets, CHK1 and 2, can both be activated by DNA damage or blocked replication [21–23](see review [24]). CHK1 and 2 are structurally related, and share significant similarities in their properties in DNA damage signaling, including many of their downstream targets [24]. However, the significant difference in their function in DNA damage response is that the *CHK1* gene knockout in mice is embryonically lethal, while the CHK2 gene knockout mice are phenotypically normal, and the CHK2$^{-/-}$ lymphocytes were remarkably resistant to IR-induced apoptosis [25–27].

Apparently based upon the knockout mice models, CHK1 is critical for cell survival and lack of CHK1 in the cells leads to cell death, while CHK2 is critical for DNA damage-induced apoptosis (cell death), and lack of CHK2 in the cells leads to cell survival (resistance to) from DNA damage-induced apoptosis (Figure 1).

It is important to remember that these regulatory molecules are present in all normal cells to protect genetic integrity [24]. However, cancer cells are not biologically normal, and it is possible that the entire signaling pathway is abnormally deleted in tumors, whereas a new pathway is abnormally developed and becomes essential for tumor cell growth. Many of the molecules in DNA damage signaling pathways are either up- or downregulated in a variety of cancer tumors. Identifying these abnormal pathways is critically important for understanding the clinical behavior of cancers and for the better design of cancer treatment.

Figure 1. The functions of CHK1 and CHK2 kinase in the DNA damage-signaling pathway.



ATR: ATM-related protein; CHK: Checkpoint kinase.

DNA damage signaling in lung cancer

Lung cancer can be classified as NSCLC and SCLC based upon histomorphology and propensity to treatment. SCLC is a neuroendocrine tumor and it is thought that SCLC is derived from neuroregulatory cells (Kulchitsky cells or K cells) scattered at the base of the normal bronchial epithelium. SCLC follows an aggressive course and it is considered a systemic disease. Surgery is not the standard option for treatment of SCLC [28]. Interestingly, SCLC shows remarkable initial response to standard chemoradiation therapy, and it rapidly develops resistance to these treatments [29]. NSCLC is a mixture of carcinomas derived from different types of cells from the bronchial–alveolar epithelium, such as squamous carcinoma, adenocarcinoma, and bronchioloalveolar carcinomas. The characteristics of these tumors to chemoradiation therapy are mixed, ranging from complete resistance to partial response [28,30]. Based upon these clinical and pathologic features, lung cancer appears to be a good model for study of both natural and acquired resistance to DNA damage-inducing agents.

We have attempted to examine the regulatory molecules in DNA damage signaling in lung cancers to see if any of these signaling molecules may have any implication in lung cancer prognosis. Surprisingly, CHK2 kinase expression was found to be significantly decreased in tumor cells of NSCLC compared with the normal lung parenchymal tissues and the bronchial epithelial cells [31]. This decrease of CHK2 kinase expression was at both the protein and the mRNA levels. CHK2 kinase was also decreased in many of the NSCLC cell lines, but not in SCLC. Furthermore, decreased expression of CHK2 mRNA in tumor cells from both the tumor tissues and the cell lines suggest a genomic or epigenetic mechanism of CHK2 gene silencing. CHK1 kinase was also examined and we did not see a significant decrease of CHK1 expression in the tumor cells [31]. This observation suggested that in NSCLC, the CHK2 signaling pathway is somewhat blocked due to decreased expression of CHK2 kinase. As suggested in the CHK2 knockout mice, lack of CHK2 kinase in normal cells was in favor of survival and rendered the cells more resistant to DNA damage-induced apoptosis (death) [32,33]. This lack of CHK2 kinase expression in NSCLC at least partially contributes to its resistance to standard therapy. Our preliminary data also suggested that *CDC25C* expression was decreased in the NSCLC cell lines [Zhang P, unpublished data], suggesting that more than one intermediate of the CHK2 signaling pathway is dysregulated in NSCLC.

Based upon the CHK2 expression status in NSCLC, we surveyed a variety of human cancer tumor samples by immunohistochemical staining and tumor-tissue microarrays (Table 1). We have found that, remarkably, NSCLC had the lowest percentage of CHK2 expression, whereas seminomatous germ-cell tumors had the highest. Other cancer types showed mixed expression of CHK2 kinase in these tumors. These tissue samples were primary resected tumors and, based upon clinical experience, the initial responses to DNA damage-inducing agents are mixed.

Another interesting observation from our laboratory is that in contrast to NSCLC in which CHK2 kinase expression was diminished, SCLC cell line H69 cells and the ovarian cancer A2780 cells expressed abundant CHK2 kinase, as demonstrated by immunoblot assays [31,34]. However, CHK2 kinase in these cells was rapidly degraded in response to cisplatin treatment. This decrease in *CHK2* expression by degradation was evident within 1 to 3 h. It is well reported that p53 stability is increased in response to DNA damage and accumulated p53 protein in the cells induces downstream targeted gene expression in regulation of the cell cycle, DNA repair and apoptosis (see review [35] and references therein). It is also well established that both CHK1 and CHK2 can activate p53 as upstream regulators through serine or threonine phosphorylations of the N- or C-terminals of p53 protein [36] (see review [24,37] and references therein). Recently, CHK2 kinase was reported as a p53-regulated gene, and downregulation of CHK2 kinase in response to cisplatin was thought to be mediated through transcriptional repression of the *CHK2* gene promoter by cooperation of p53 with other transcriptional factors [38]. We have demonstrated that CHK2 protein that *CHK2* mRNA expression was not affected by actinomycin D in response to cisplatin. We showed that downregulation of CHK2 kinase expression in response to cisplatin was at protein levels and the degradation of CHK2 kinase can be partially blocked by adding the proteasome inhibitor MG132, suggesting the important role of proteasome in the degrading process. It is possible that p53 status in the cells influences

Table 1. Tumor tissue microarray and immunohistochemical study of CHK2 kinase expression in common cancers.

Cancers	CHK2 expression in common cancers	
	Positive (%)	***Negative (%)***
Lung (n = 92)	17 (16/92)	83 (76/92)
Breast (n = 130)	48 (63/130)	52 (67/130)
Ovary (n = 43)	49 (21/43)	51 (22/43)
Colon (n = 93)	76 (71/93)	24 (22/93)
Prostate (n = 87)	76 (66/87)	24 (21/87)
Seminoma (n = 25)	88 (22/25)	12 (3/25)

n: Number of tumor cases.

CHK2 degradation, since in the OV3 ovarian cancer cells, another ovarian cancer cell line in which the p53 gene is known to be mutated, CHK2 degradation was significantly reduced [Zhang P, unpublished data]. It is also likely that both transcriptional repression of CHK2 gene expression by p53 induced by cisplatin and degradation of CHK2 at the protein level contribute to the diminished level of CHK2 in the cells, but the relative contribution from each mechanism remains to be established.

We took advantage of a pair of ovarian cancer cell lines, cisplatin-sensitive A2780 cells and cisplatin-resistant CP70 cells. CP70 cells were derived from A2780 cells by repeated exposure to increasing doses of cisplatin [39]. We have found that in CP70 cells, CHK2 kinase expression was markedly reduced in comparison with that in A2780 cells. These data suggest that CHK2 degradation in response to cisplatin appears to be a survival signal for the cancer cells not to commit DNA damage-induced apoptosis, further supporting the important role of CHK2 kinase in apoptosis discovered in the knockout mouse model.

Degradation of CHK2 kinase in response to cisplatin in SCLC and ovarian cancers is not unique in these cell lines. We have tested other cell lines, such as squamous carcinoma cells of the oral cavity (SCC-015) and the immortalized transformed normal bronchial epithelial cells (BEA-S). CHK2 kinase was also degraded in these cells in response to cisplatin [40]. Furthermore, CHK2 kinase was degraded in response to other DNA damage-inducing agents, such as UV and IR. Degradation of C+HK2 can occur within 1 h in response to high doses of cisplatin. Taken together, these data suggest that DNA damage can induce CHK2 degradation, and degradation is a central part of DNA damage signaling for cell survival.

Similar protein degradation in response to DNA damage was observed for CDC25A, a critical mediator in the DNA damage-signaling pathway [41,42]. CDC25A is a downstream target for both CHK1 and CHK2 kinases. Contribution of CDC25A degradation in response to DNA damage to tumor resistance is yet to be determined.

DNA damage signaling, cancer drug targets & cancer clinical trials

Discovery of CHK1 and 2 kinase as important intermediaries in DNA damage signaling suggests that these kinases can be targets for drug development. A specific inhibitor of CHK1 kinase is potentially useful in the treatment of NSCLC, since a lack of CHK1 kinase activity is biologically lethal, whereas maintaining CHK2 kinase activity in cancer cells by blocking CHK2 degradation through proteasome activity will sensitize these cells to DNA damage-inducing cytotoxic agents. Currently, a number of biopharmaceutical companies are actively developing CHK1 kinase inhibitors. UCN-01, a specific protein kinase C inhibitor, was found to also inhibit the CHK1, CHK2 kinases as well as cyclin-dependent kinase [43]. UCN-01 was tested clinically and it appeared to bind to plasma protein with high affinity and it was this binding that seemed to affect the bioavailability of the drug to the tumor cells. Despite a number of shortcomings, UCN-01 is at various stages of clinical trials for malignant solid tumors, such as renal cell carcinoma, sarcomas and lymphomas. Other companies are now actively engaged in the discovery of more specific inhibitors of CHK1 kinase. Based upon our results for NSCLC and CHK2 kinase expression, we believe that a specific CHK1 inhibitor would be potentially useful for treatment of NSCLC.

Highlights

- Lung cancer is notoriously resistant to DNA damage-inducing therapies, such as cytotoxic chemotherapy and radiation therapy.
- Discovery of alterations of DNA damage signaling pathway in cancer cells provides new direction of research to cancer resistance.
- Key intermediaries in the DNA damage-signaling pathway may serve as potential targets for drug discovery.

Expert opinion

It is worth noting that there are various clinical trials currently underway for the proteasome inhibitors (i.e., Velcade and PS-341) in the treatment of solid tumors, in addition to multiple myeloma [44,45]. Our prediction is that the proteasome inhibitors would be more potent when combined with cytotoxic agents, such as cisplatin or radiation. A synergistic effect exists between cisplatin and MG132 for the killing of the H69 SCLC cells and A2780 ovarian cancer cells [Zhang P, unpublished data]. It is difficult to envision that the proteasome inhibitors can work alone to induce cell death based on the cell culture study, and it may be more fruitful when combined with other existing cytotoxic DNA damage-inducing agents in clinical trials.

Outlook

A number of specific inhibitors targeting the intermediaries of DNA damage-signaling pathways are at various stages of developments, and these inhibitors are likely to have significant impact in cancer treatment, especially lung cancer; the leading killer of all cancer patients.

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Affiliation

Peilin Zhang, MD, PhD
Putnam General Hospital,
Hurricane, WV 25526, USA
Tel.: +1 304 757 1770
Fax: +1 304 757 1736
Peilin.Zhang@hcahealthcare.com