Role of cell cycle regulators in lung carcinogenesis

Beatrice Eymin and Sylvie Gazzeri*
Equipe Bases Moléculaires de la Progression des Cancers du Poumon; Centre de Recherche INSERM U823; Institut Albert Bonniot; Grenoble, France; and Université Joseph Fourier; Grenoble, France

Key words: lung cancer, cell cycle regulators, kinases, checkpoint, therapy

Overview of the Cell Cycle Regulation

The mammalian cell cycle machinery is comprised of five sequential stages: G0, G1, S, G2, and M. During two of these phases, cells execute the two basic events in cell division: generation of a single and faithful copy of its genetic material (S phase) and partitioning of all the cellular components between two identical daughter cells (mitosis or M phase). The two other phases of the cell cycle, G1 and G2, represent “gap” periods during which cells prepare themselves for the successful completion of the S and M phases respectively. When cells cease proliferation, either due to specific antimitogenic signals or to the absence of proper mitogenic signalling, they exit the cycle and enter a non-dividing quiescent state known as G0. These last decades, the molecules controlling the successive phases of the cell cycle have been extensively characterized. The central players are the cyclin-dependent kinases (CDKs), a group of serine/threonine kinases whose activity is regulated by their arrangement in a multimeric complex with larger proteins called cyclins, owing to their cyclical expression and proteasomal degradation during the cell cycle. Throughout the cell cycle progression, different CDK-cyclin complexes are formed and are activated by sequential phosphorylation/dephosphorylation with a clear-cut timing, thereby preventing cells from entering into a new phase until they have successfully completed the previous one.2 In addition, all along the cell cycle, a series of surveillance pathways named cell cycle checkpoints ensure that cells pass accurate copies of their genome onto the next generation, in order to preserve genome integrity and chromosomal stability.

The Molecular Players of G1 and S Phases

When cells in the quiescent (G0) phase enter the cycle in response to extracellular signals such as mitogenic activation, intracellular levels of D-type cyclins (D1, D2, D3) increase, resulting in the formation and nuclear localization of cyclin D-CDK4 and cyclin D-CDK6 complexes that initiate phosphorylation of the retinoblastoma protein (RB1, also known as p105-RB) and possibly of other members of the “pocket” protein family.3 4 The RB pocket
proteins (p105, p107 and p130) negatively modulate the G1 to S phase transition at least in part through binding and inactivation of the E2F transcription factors that promote transcription of genes required for DNA replication. Partial phosphorylation of RB proteins by cyclin D-CDK4 and cyclin D-CDK6 complexes in early G1 inactivates their function as transcriptional repressors and leads to the release of E2F transcription factors, enabling the expression of genes required for G1 to S phase transition and DNA synthesis. In late G1, levels of E-type cyclins accumulate and these cyclins associate with CDK2 to reinforce RB1 phosphorylation on additional sites, and to irreversibly initiate the gene expression programme of the S phase. This stage, called the restriction point, is crucial in cancer as alterations of the molecular players involved in the G1 to S phase transition allow cells to proliferate independently of mitogenic stimuli. It is therefore not surprising that CDK inhibitors (CKI) exist to control the timely activation of cyclin-CDK complexes. These proteins belong to two different families: the INK4 family of proteins that include INK4A (also known as p16^INK4a_), INK4B (also known as p15^INK4b_), INK4C (also known as p18^INK4c_), and INK4D (also known as p19^INK4d_), as well as the kinase inhibitory protein (WAF/KIP) family. The four members of the INK4A family exert their inhibitory activity by binding to the CDK4 and CDK6 kinases and by preventing their association with D-type cyclins. The three members of the WAF/KIP family, WAF1 (also known as p21^WAF1/CIP1_), KIP1 (also known as p27^KIP1_) and KIP2 (also known as p57^KIP2_) can form heterotrimetric complexes with the G1/S CDKs. However, in stoichiometric amounts, they only inhibit the kinase activity of cyclin E-CDK2 complexes. Beyond the restriction point, RB1 is maintained in a hyperphosphorylated state through the sequential activities of cyclin A-CDK2, cyclin A-CDK1 and cyclin B-CDK1 complexes, thereby ensuring S phase completion.

The Molecular Players of the G2 and M Phases

In parallel with DNA replication, the centrosome cycle begins. Centrosomes duplicate during late S phase to early G2 phase, and separate to form the poles of the mitotic spindle at the beginning of mitosis. At these poles, each centrosome matures to form its own aster of dynamic microtubules. The centrosome cycle and the formation of the mitotic spindle are controlled by mitotic kinases such as CDK1. During the G2/M transition, the cyclin A-CDK1 complex is activated to initiate mitosis through regulation of chromosome condensation and microtubule dynamics. Then, following destruction of the nuclear membrane, cyclin A is degraded and cyclin B1-CDK1 complexes are activated to allow the progression through the M phase by promoting chromosome condensation and spindle assembly. The complete separation of the two daughter cells occur when cyclin B1 is degraded by the anaphase-promoting complex or cyclosome (APC-C). Aurora and polo-like kinase (PLK) are also essential regulators of mitosis. Aurora A localizes to duplicated centrosomes and spindle poles during mitosis and has well-established roles in centrosome function and duplication, mitotic entry and bipolar spindle assembly. Aurora B is the catalytic component of the chromosomal passenger complex, which is composed of three additional noncatalytic subunits that direct its activity: survivin, INCENP and borealin. The chromosomal passenger complex orchestrates the accurate segregation of the chromatids, histone modification and cytokinesis. Aurora C does not seem to have a role in mitosis in the majority of normal cells, with expression essentially restricted to the testis. The best characterized member of the mammalian Polo-like family is PLK1. PLK1 specifically localizes to centrosomes, the spindle midzone and the post-mitotic bridge, and participates in both mitotic entry and mitotic progression.

The Regulators of the Cell Cycle Checkpoints

The cell cycle checkpoints are designed to preserve genome integrity and chromosomal stability in response to induced or spontaneous DNA lesions that are common events in the life of the cell, as well as upon abnormal chromosomal segregation. They constitute therefore a physiological barrier that guards against progression of tumors from early stages to malignant invasive lesions. Upon DNA damage, these checkpoints give the cell time to repair the DNA lesion by triggering cell cycle arrest in G1, S or G2 phase. If lesions are irreparable, the programmed cell death is induced. The crucial regulators of these pathways are the related kinases ataxia telangiectasia mutated (ATM), ataxia telangiectasia and RAD3-related protein (ATR) and their downstream effectors, the checkpoint kinases CHK1 and CHK2. The ATM/CHK2 pathway regulates mostly the G1 checkpoint, through activation of the tumor suppressor gene p53, leading to transcriptional activation of the CDK1 p21^WAF1/CIP1_ that prevents cells from entering the S phase. Damaged cells that have already passed the transition from G1 to S phase can also be halted before entry into mitosis through activation of the ATR/CHK1 pathway that induces the cytoplasmic sequestration of the CDC25C phosphatase required for CDK1 activation. Lastly, the mitotic checkpoint, also known as the spindle assembly checkpoint (SAC) is activated when chromosomes are not properly attached to the mitotic spindle. This pathway involves the activation of signalling proteins including aurora B, mitotic arrest deficient proteins 1 and 2 (MAD1, MAD2), monopolar spindle 1 (MPS1), budding uninhibited by benzimidazole 1 (BUB1) and its homologous BUB3 and BUB1B. They act by inactivating APC-C, thereby preventing cyclin B1 proteolysis and cytokinesis. These last decades a growing number of studies have demonstrated the constant invalidation of some of these cell cycle regulators (Fig. 1) and checkpoint proteins (Fig. 2) in human lung tumors. In the second part of this review, we will summarize the principal points of deregulation according to each phase of the cell cycle.

Alteration of the Components of the G1 to S Phase Transition in Lung Cancers

The p16^INK4A/cyclin D1/CDK4-CDK6/RB pathway. The retinoblastoma gene, located on chromosome 13q14, was the first tumor suppressor identified in lung cancer. Loss of RB1 protein
is observed in a high proportion of SCLC tumors (>90%) as a
dresult of loss of heterozygosity (LOH) at the RB locus and inactiva-
tion of the remaining allele by several mechanisms including point
mutations or decreased mRNA expression. By contrast, loss of RB1 protein occurs in 15% of NSCLC, and RB2/ p130 point mutations are also observed in primary NSCLC and
undetectable levels of RB2/p130 protein are associated with the
most aggressive tumor phenotypes, suggesting an independent
role of this pocket member in the development and/or progression
of NSCLC. In SCLC, dysregulation of p107 and p130 has been rarely reported.

In contrast to neuroendocrine lung tumors in which RB protein
is frequently lost, the majority of NSCLC exhibits RB inactiva-
tion through the deregulation of upstream regulators of RB pathway,
such as p16INK4a and cyclin D1. These events lead “in fine”
to RB1 hyperphosphorylation and loss of anti-proliferative con-
trol. Loss of p16INK4a protein is observed in 40–50% of NSCLC
as a consequence of gene deletion, promoter hypermethylation or gene mutation. Homozygous deletions of the INK4a locus
occurs in 30% of NSCLC tumors whereas inactivating mutations of INK4a gene are relatively rare. Hypermethylation of the
INK4a promoter is observed in about 40% of the cases and is also
detected in bronchial epithelium from chronic smokers, suggesting
that inactivation of p16INK4a is an early event in lung tumorigenesis. Studies evaluating the effect of p16INK4a expression on
prognosis have shown an improved survival of NSCLC patients
with high p16INK4a levels, although not all studies have reached
statistical significance. Amplification of the cyclin D1 locus is
observed in 5–32% of tumors depending on the studies. High
levels of the cyclin D1 protein are found in invasive NSCLC as
well as in a significant fraction of non-invasive lesions of the bron-
chial epithelia, indicating that, like p16INK4a, increased cyclin
D1 expression is an early event. Overexpression of cyclin D1 is
associated with a more favorable clinical outcome in some stud-
ies, whereas others correlate the upregulation of cyclin D1 with a
worse outcome or even do not find any association. In a study
limited to stages I and II NSCLC in which cyclin D1 expression
was associated with shorter survival, a combination of high cyclin
D1 levels with loss of p16INK4a was observed in patients with the
worst prognosis. In contrast to p16INK4a or cyclin D1 dysregula-
tion, amplification of CDK4 gene leading to CDK4 overexpression
is a rare event in NSCLC. As a whole, these studies demonstrate
that inactivation of the RB pathway is a constant event in lung
cancer. In NSCLC, p16INK4a loss and cyclin D1 overexpression are
always inversely correlated with RB loss indicating that cyclin D1
and p16INK4a act only through RB pathway during lung carcino-
genesis. This is also the case in SCLC in which RB is mostly lost
and p16INK4a or cyclin D1 alterations are rare events.

The three other members of the CDKI INK4 family:
• p15INK4b, p18INK4c, p19INK4d. The cell cycle inhibitor p15INK4b is
frequently inactivated by homozygous deletions in NSCLC, always
together with p16INK4a. In contrast, homozygous deletions of...
The status of p19INK4d has never been addressed in lung tumors.

The CIP/KIP family. The CDK inhibitor p21WAF1/CIP1 inhibits the progression through the cell cycle via several mechanisms including inhibition of the cyclinD1/CDK4 and cyclin E/CDK2 complexes in early G, and inhibition of the cyclinA/CDK2 complex prior to the S/G, transition. In NSCLC, a positive expression of p21WAF1/CIP1 is detected more frequently in patients with stage I or II disease than in those with stage IIIa disease.53,54 In multivariate analyses, patients with tumors expressing p21WAF1/CIP1 survive longer than do those with tumors negative for p21WAF1/CIP1 expression.53,54 Therefore, positive expression of p21WAF1/CIP1 appears to be a significant factor for predicting a favorable prognosis. In addition, NSCLC patients who are negative for both p21WAF1/CIP1 and p16INK4a proteins have a significantly shorter overall survival,53 indicating that both CDKIs do not act on the same targets to inhibit lung tumorigenesis.

p27KIP1 is also a strong inhibitor of cell cycle progression through its ability to inhibit cyclin D/CDK4, cyclin D/CDK6, cyclin E/CDK2 and cyclinA/CDK2 complexes. Low levels of p27KIP1 are observed in NSCLC as compared to normal counterparts, and correlate with reduced cancer cell differentiation66,67 and high proliferative index.56 Downregulation of p27KIP1 associates with a poor outcome in NSCLC patients and is a significant prognostic factor in multivariate analyses.58-61 In contrast, SCLC exhibit increased p27KIP1 expression when compared to the normal lung epithelium.61 Together, these data suggest that p27KIP1 might play distinct biological roles in the pathogenesis of SCLC and NSCLC. In favor of such hypothesis, overexpression of p27KIP1 in SCLC cell lines has been reported to protect the cells from apoptosis in unfavourable microenvironments.62 In NSCLC, the low levels of p27KIP12 are associated with a high p27KIP1 proteolytic activity.63 p27KIP1 degradation is mediated at least in part by SKP2, an F-box-protein of the SCF complex. SKP2 has oncogenic properties and increased SKP2 protein levels have been observed in lung cancers of all histological types.64,65 Although low level of p27KIP1 do not always correlate with high SKP2 overexpression, high SKP2 protein levels have been associated with reduced p27KIP1 expression in NSCLC suggesting that dysregulation of SKP2 contributes to the altered expression of p27KIP1 in that case.66 In contrast, we showed that SKP2 and p27KIP1 are directly correlated in NE lung tumors85 confirming that both proteins might have distinct functions according to the histological types of lung tumors.

Only few studies have investigated the status of p57KIP2 in lung tumors. A significant reduced expression of p57KIP2 associated with a decrease of p27KIP1 has been reported in NSCLC as compared to normal counterparts, and was correlated with increased cellular proliferation.66 For both KIPs, SKP2-mediated
proteolysis was the most important mechanism for downregulation although correlation between decreased level of p57KIP2 mRNA and promoter methylation, allelic loss or imprinting has been reported in some cases. Ablant methylation of p57KIP2 promoter has been also observed in another study on lung cancer cell lines and tumors. As p57KIP2 expression could be restored in methylated cell lines following 5-aza-2-deoxycytidine treatment, these data indicate that methylation contributes to p57KIP2 inactivation in lung cancer.

The transcription factors of E2F family. It is currently admitted that loss of RB function contributes to uncontrolled cell proliferation by unleashing E2F transcription factors activity. In this respect, abnormal expression of the E2F1-E2F3 proteins is observed in a growing number of tumors. We previously showed that E2F1 is overexpressed in SCLC while it is undetectable in NSCLC as compared to corresponding normal lung, thereby identifying a differential pattern of E2F1 protein expression in lung tumors. By contrast, other studies have reported amplification of the E2F1 gene locus at 20q11.2,70 as well as increased E2F1 protein level in NSCLC,71,72 and have shown that E2F1 is an adverse prognostic factor in these tumors. The reasons of such discrepancies remain unknown. More recently, E2F1 expression was reported to correlate with expression of its transcriptional targets thymidilate synthase and survivin in NSCLC and to was reported to correlate with expression of its transcriptional activator E2F1. E2F1 expression is an early event during lung tumorigenesis.40 Cyclin E expres-

Alteration of the Components of G1 Phase in Lung Tumors

The progression through S phase is principally regulated by the cyclin A/CDK2 complex. Elevated levels of cyclin A are observed in primary NSCLC, in lymph node metastasis and in some bronchial precursor lesions as compared to normal bronchial epithelium. Several studies have reported that overexpression of cyclin A is consistently associated with an unfavourable outcome in patients with NSCLC. Moreover, elevated levels of the licensing factors hCdt1 and hCdc6 that are key elements for DNA replication, are observed in NSCLC and correlate to increased tumor growth and aneuploidy in p53-defective tumors.

Alteration of the Components of G2 and M Phases in Lung Tumors

CyclinB1/CDK1 is the classic M phase-promoting factor that drives entry into mitosis. High levels of cyclin B1 are observed in NSCLC, especially in stage I squamous cell cancers. Increased expression of cyclin B1 is also detected in some precursor lesions. High cyclin B1 expression correlates with differentiation, invasion and high proliferative index.53,86,88 Cyclin B1 has also been reported as a significant prognostic factor in NSCLC in multivariate analysis. In other studies, cyclin B1 was not found to be an independent prognostic parameter although its overexpression seems to be an adverse prognostic factor. Elevated levels of cyclin B1 have been associated with poor outcome in patients with early stage squamous cell carcinoma of the lung, suggesting that cyclin B1 expression may be a prognostic marker for these patients. Although the role of cyclin B1 in lung tumor development has been the subject of many studies, the implication of CDK1 has not been properly evaluated. However, in a genome profiling study high levels of CDK1 have been reported in early stage lung adenocarcinoma.90 CDK1 activity is controlled by phosphorylation, a process finely regulated by the WEE1 and PLK1 kinases. WEE1 delays mitosis by suppressing the activity of the cyclinB1/CDK1 complex whereas PLK1 favors mitosis by allowing the activation of cyclinB1/CDK1 complex. Downregulation of WEE1 expression has been reported in lung tumors. Patients with lack of WEE1 have a higher recurrence rate and a poorer prognosis and WEE1 expression is a significant prognostic factor in multivariate analysis. Therefore, the loss of WEE1 may have a potential role in promoting tumor progression and may be a significant prognostic indicator in NSCLC. By contrast, elevated levels of PLK1 are observed in NSCLC and overexpression of PLK1 is a negative prognostic factor in NSCLC patients. Interestingly, mice heterozygous for PLK4, another member of PLK family involved in centrosome separation and mitotic fidelity, develop lung tumors due to high frequency of
mitotic errors.\textsuperscript{94} Although the status of PLK4 has not been yet studied in lung tumors, these data suggest that PLKs might have opposite roles in lung tumorigenesis.

As defects in mitosis can lead to genomic instability, deregulation of the expression and activity of Aurora kinase family members has been implicated in tumorigenesis. A certain number of studies indicate that dysregulation of Aurora kinase plays a key role during lung carcinogenesis. Overexpression of \textit{Aurora A} transcript and protein has been reported in NSCLC as compared to normal lung tissue and was correlated with poor differentiation.\textsuperscript{92} Association between \textit{Aurora A} polymorphisms and lung cancer risk has also been described.\textsuperscript{93} Moreover, a general upregulation of \textit{Aurora B} mRNA levels has been observed in NSCLC compared with normal epithelium, and was correlated with the level of genetic instability of the tumors.\textsuperscript{94,95} Overexpression of \textit{Aurora B} protein has also been found in NSCLC and was significantly correlated with expression of survivin, a component of the chromosomal passenger complex.\textsuperscript{96} In this study high \textit{Aurora B} expression levels were significantly associated with squamous cell carcinoma histology, poor tumor differentiation and lymph node invasion and predicted shorter survival for the patients with adenocarcinoma histology.

Although less extensively studied than \textit{Aurora} genes, other mitotic genes display lung cancer-associated altered expression. They include microtubule-associated proteins such as TPX2 and TACC3 which overexpression has been associated with poor clinical outcome.\textsuperscript{97,98} Another example is the APC/C activator, CDC20, which upregulation has been observed in early stage lung adenocarcinoma.\textsuperscript{89}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Alterations of the G1/S transition regulators in NSCLC versus SCLC. Disruption of the G1/S transition is a crucial event during lung carcinogenesis. Striking differences are observed regarding the mechanisms of G1 escape in NSCLC versus SCLC. Pale grey circles indicate loss of expression of the corresponding protein and dark grey circles indicate overexpression. E2F1 and p15\textsuperscript{INK4b} are depicted by gradual color in NSCLC and SCLC respectively, as controversial results have been reported regarding their status in these tumors. In all other cases, abnormalities are very infrequent or have not been studied. The percentage of dysregulation is presented next to each molecule.}
\end{figure}

\textbf{Alteration of the Components of Cell Cycle Checkpoints in Lung Tumors}

The cell cycle checkpoints are designed to preserve genome integrity. Therefore, abnormalities of components of these networks likely play a role in the development of tumors. For instance, mutations and methylation that reduce activity of CHFR, a mitotic checkpoint gene that delays chromosome condensation in response to microtubule poisons have been described in NSCLC.\textsuperscript{91} Although the status of PLK4 has not been studied in lung tumors, these data suggest that PLKs might have opposite roles in lung tumorigenesis.
As discussed above, CDKs are often overactive in lung cancer resulting in loss of checkpoint integrity and uncontrolled proliferation. Therefore, selective inhibitors of CDKs may limit the progression of a tumor cell through the cell cycle and facilitate the induction of apoptotic pathways. The therapeutic value of small molecules CDK inhibitors that modulate CDK by competing with ATP binding is the subject of intense work. First generation compounds to be evaluated in clinical trials included the pan-CDK inhibitor flavopiridol which induced partial responses or stable disease in patients with NSCLC in a phase I study, when using in combination with the cytotoxic agents paclitaxel and carboplatin. A second-generation CDK inhibitor, the aminothiazole SNS-032, which was recently shown to sensitize radiotherapy-resistant NSCLC cells to ionizing radiation, is currently in phase II clinical trials with irinotecan and SCH727965 which is in phase II trial. Of note Indisulam is not a direct CDK inhibitor but it cause a depletion of cyclin E which reduces CDK2 activity.

**Cell Cycle Regulators as Targets for Lung Cancer Therapy**

Aberrations in cell cycle control are a hallmark of lung tumors. Therefore, modulation of cell cycle regulators may have an important use for the treatment of these cancers. In last part, we discuss the strategies that are currently developed to target the cell cycle machinery in lung tumors (Table 1).

**Inhibitors of cyclin-dependent kinases.** As discussed above, CDKs are often overactive in lung cancer resulting in loss of checkpoint integrity and uncontrolled proliferation. Therefore, selective inhibitors of CDKs may limit the progression of a tumor cell through the cell cycle and facilitate the induction of apoptotic pathways. The therapeutic value of small molecules CDK inhibitors that modulate CDK by competing with ATP binding is the subject of intense work. First generation compounds to be evaluated in clinical trials included the pan-CDK inhibitor flavopiridol which induced partial responses or stable disease in patients with NSCLC in a phase I study, when using in combination with the cytotoxic agents paclitaxel and carboplatin. A second-generation CDK inhibitor, the aminothiazole SNS-032, which was recently shown to sensitize radiotherapy-resistant NSCLC cells to ionizing radiation, is currently in phase II clinical trials with irinotecan and SCH727965 which is in phase II trial. Of note Indisulam is not a direct CDK inhibitor but it cause a depletion of cyclin E which reduces CDK2 activity.

**Inhibitors of mitotic checkpoint kinases.** Many patients with cancer receive antimitotic agents that act as microtubule toxins as first-line therapy. However, because of the side effects of these drugs, these last years much work has focused on the identification of new mitotic targets that could block spindle assembly without affecting microtubules. In this respect, aurora kinases and PLKs have received particular attention and a diverse array of inhibitors have been developed. Preliminary clinical data from phase I trials have largely been consistent with cytostatic effects, with disease stabilization as the best response achieved in solid tumors. As an example, the pan-aurora inhibitor MK-0457 (VX-680) blocks tumor xenograft growth and induces tumor regressions in preclinical models. In phase I-II trials, MK-0457 was given to patients with previously treated tumors and disease stabilization.

**Table 1. Selected inhibitors of cell cycle regulators used in clinical trials for lung cancer therapy**

| Inhibitor | Main targets | Clinical trials |
|-----------|-------------|-----------------|
| **Inhibitors of cyclin-dependent kinases** | | |
| Flavopiridol also known as alvocidib | CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 | Phase I: NSCLC in combination with paclitaxel and carboplatin (ref. 115) |
| Aminothiazole SNS-032 also known as BMS-387032 (Sunesis) | CDK2, CDK7 and CDK9 (CDK1 and CDK4) | Phase I: NSCLC Sensitized radioresistant NSCLC cells to ionizing radiations (ref. 116) |
| R-roscovitine also known as CYC202 and seliciclib (Cyclacel) | CDK1, CDK2, CDK5, CDK7 and CDK9 | Phase I-II: NSCLC (ref. 117) |
| Indisulam, also known as E7070 SCH 727965 | Not Assigned | Phase I: lung cancer in combination with irinotecan Phase II: NSCLC |
| VX-680, also known as MK-0457 | Pan-aurora | Phase I-II: NSCLC Trials discontinued owing to QT prolongation (ref. 118) |
| **Inhibitors of DNA Damage Checkpoint kinases** | | |
| 7-hydroxy-staurosporine, also known as UCN-01 | CHK1 and MARK3 (PKC, PKD1, GSK3β, CDK1, CDK2 and CHK2) | Phase II: SCLC (with topotecan) |
was observed in one patient with lung tumor. However, owing to QT prolongation in 1 in 100 patients, trials have been discontinued. Numerous other compounds targeting Aurora and PLK kinases are currently in clinical development. Their use in lung tumors therapy has not been yet evaluated.

**Inhibitors of DNA damage checkpoint kinases.** Two small molecules ATM inhibitors have been described (KU55933 and CP466722) that target ATM by blocking its ATP-binding site and display high specificity. Both compounds prevent phosphorylation of ATM effectors and sensitize cells to drugs that induce DNA double-strand breaks. They also specifically and reversibly disrupt ATM dependent cell cycle checkpoint in response to DNA damage induced by ionizing radiation. Although this has to be fully investigated “in vivo,” the “in vitro” effects of these novel lead chemotypes are promising. At present, no specific ATR inhibitors have been identified. However, several CHK1 and CHK2 inhibitors have been developed and are currently in clinical evaluation. One of them, 7-hydroxystaurosporine (UCN-01) is undergoing Phase II trials in SCLC patients in combination with topotecan cytotoxic agent. This compound efficiently abrogates DNA damage checkpoint in cancer cells that lack p53 and have been treated with DNA-damaging drugs, resulting in mitotic catastrophe.

**Concluding Remarks**

These last decades, numerous studies have been conducted to examine the status of cell cycle regulators in human lung tumors. They have led to the conclusion that all the signaling networks controlling cell cycle progression are deregulated in these cancers. Based on these studies, independent prognostic factors have been identified that may be important in predicting patient outcome as well as clinical response to therapy. In addition, potential therapeutic targets have been identified and several therapeutic approaches have been developed. In this setting, the search of synthetic inhibitors of cyclin-dependent kinase as anticancer drugs is currently a growing field of research, and new generation of CDK inhibitors with superior activity and specificity is the subject of intense exploration. Moreover, there have been recent advances in the development of drugs that target checkpoints and mitotic kinases, and several ATP-competitive inhibitors are currently in clinical evaluation. Current research goals also include combination of cell cycle kinases inhibitors with classical chemotherapy to enhance clinical efficacy. Indeed, in most human lung tumors, the function of the DNA damage checkpoint in G1 is impaired owing to the loss of p53/ RB function. Treatment of these cells with chemotherapeutic agents often results in S or G2 checkpoint-mediated arrest. Therefore, abrogation of these DNA damage checkpoints is an attractive strategy currently being explored in chemotherapeutic-combined clinical trials as it could result in mitotic catastrophe and cell death. In addition, inhibitors targeting Aurora kinases have given very promising results and the knock-down of Aurora A by RNA interference have been found to sensitize lung cancer cells to chemotherapy. Further studies need to be elucidated by the mechanisms which target Aurora kinase interacts with chemotherapy at the molecular and cell cycle levels but these results are very encouraging. Moreover, as cell cycle regulators are also involved in important molecular pathways with therapeutic cues in lung cancer (EGFR, Kras, Braf, ...), uncovering the potential usefulness of combined therapies would probably provide novel insights into lung cancer treatment.

**Acknowledgments**

Supported by the Ligue Nationale contre le Cancer (Equipe labellisée Ligue 2007), the Conseil Scientifique National d’AGIR à dom. and INCa (Programme National d’Excellence Spécialisé, 2005–2007).

**References**

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.
2. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science 1989; 246:629-34.
3. Giacinti C, Giordano A. RB and cell cycle progression. Oncogene 2006; 25:5220-7.
4. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995; 81:323-30.
5. Lam EW, La Thangue NB. DP and E2F proteins: coordinating transcription with cell cycle progression. Curr Opin Cell Biol 1994; 6:859-66.
6. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 1999; 98: 859-69.
7. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1 phase progression. Gen Dev 1999; 13:1501-12.
8. Malumbres M, Barbacid M. Mammalian cyclin-dependant kinases. Trends Biochem Sci 2005; 30:630-41.
9. Barr AR, Gergele F. Aurora-A: the maker and breaker of spindle poles. J Cell Sci 2007; 120:2987-96.
10. Archambault V, Glover DM. Polo-like kinases: conservation and divergence in their functions and regulation. Nat Rev Mol Cell Biol 2009; 10:265-75.
11. Marumoto T, Zhang D, Saya H. Aurora-A—a guardian of poles. Nat Rev Cancer 2005; 5:42-50.
12. Vider G, Medema RH, Lenu SM. The chromosomal passenger complex: guiding Aurora-B through mitosis. J Cell Biol 2006; 173:833-7.
13. Tang CJ, Lin CY, Tang TK. Dynamic localization and functional implications of Aurora-C kinase during male meiosis. Dev Biol 2006; 290:398-410.
14. Sasaki K, Karayama H, Sueno DL, Fujii S, Honda R, Kimura M, et al. Aurora-C kinase is a novel chromosomal passenger protein that can complement Aurora-B kinase function in mitotic cells. Cell Motil Cytoskeleton 2004; 59:249-63.
15. Strebhardt K, Ullrich A. Targeting polo-like kinase 1 for cancer therapy. Nat Rev Cancer 2006; 6:321-30.
16. Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division. Nat Rev Mol Cell Biol 2004; 5:429-40.
17. Barthova J, Hoerejz Z, Koud K, Kramer A, Tort F, Zieger K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005; 434:864-70.
18. Gorgoulis VG, Vasilieou LV, Karakaidos P, Zacharatos P, Kotsinas A, Lioglio T, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005; 434:907-13.
19. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 2003; 3:155-68.
20. Yokota J, Morit N, Akiyama T, Shimosato Y, Sugimura T, Terada M. Multiple genetic alterations in small-cell lung carcinoma. Princess Takamatsu Symp 1989; 20:43-8.
21. Harbour JW, Lai SL, Whang-Peng J, Gazdar AF, Minna JD, Kaye FJ. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 1988; 241:353-7.
22. Gouyer V, Gazzini S, Bolon I, Drever C, Brambilla C, Brambilla E. Mechanism of retinoblastoma gene inactivation in the spectrum of neuroendocrine lung tumors. Am J Respir Cell Mol Biol 1998; 18:188-96.
23. Gouyer V, Gazzini S, Brambilla E, Bolon I, Moro D, Perron P, et al. Loss of heterozygosity at the RB locus correlates with loss of RB protein in primary malignant neuro-endocrine lung carcinomas. Int J Cancer 1994; 58:818-24.
24. Haga Y, Hiroshima K, Iyoda A, Shibuuya K, Shimamura F, Itazu T, et al. Ki-67 expression and prognosis for smokers with resected stage I non-small cell lung cancer. Ann Thorac Surg 2003; 75:1727-32.
25. Reissmann PT, Koga H, Takahashi R, Figlin RA, Holmes EC, Piantadosi S, et al. Inactivation of the retinoblastoma susceptibility gene in non-small-cell lung cancer. The Lung Cancer Study Group. Oncogene 1993; 8:1913-9.
26. D’Amico TA, Massey M, Herndon JE, 2nd, Moore MB, Harpole DH Jr. A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of ten molecular markers. J Thorac Cardiovasc Surg 1999; 117:76-83.

27. Bardele PP, Howard CM, Paclitaxel C, Ciriolo C, Romano G, Minisini C, et al. Mutations in the retinoblastoma-related gene RB2/p130 in lung tumors and suppression of tumor growth in vivo by retrovirus-mediated gene transfer. Cancer Res 2000; 60:372-82.

28. Varhelyi E, Esposito V, De Luca A, Fu Y, Meiari L, Giordano GC, et al. Differential expression of RB2/p130 and p107 in normal human tissues and in primary lung cancer. Clin Cancer Res 1997; 3:1691-7.

29. Baldi A, Esposito V, De Luca A, Howard CM, Mazzarella G, Baldi F, et al. Differential expression of the retinoblastoma gene family members pRb/p105, p107 and pRb2/p130 in lung cancer. Clin Cancer Res 1996; 2:1239-45.

30. Helin K, Holm K, Niebuhr A, Eiberg H, Tommerup N, Hougaard S, et al. Loss of the retinoblastoma protein-related p130 protein in small cell lung carcinoma. Proc Natl Acad Sci USA 1997; 94:6933-8.

31. Shapiro GI, Edwards CD, Kobzik L, Godleski J, Pellegrini S, Gaeta P, et al. Cyclin D1 and retinoblastoma-related gene RB2/p130 in non-small cell lung cancer at stages I and II. Lung Cancer 2003; 55:1-14.

32. Gazzeri S, Gouyer V, Vour’ch C, Brambilla C, Brambilla E. Mechanisms of p16 INK4A inactivation in non small-cell lung cancers. Oncogene 1998; 16:497-504.

33. Kallioniemi A, Salmenkivi K, et al. CDK4 is a putative tumor suppressor gene. Cancer Res 1997; 57:58-63.

34. Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Takahashi S, Kamata Y, Tamo W, Koyanagi M, et al. Overexpression of cyclin D1 (CCND1) in squamous cell lung cancer: an exit from cell cycle control. Nat Rev Cancer 2005; 9:785-97.

35. Eymin B, Gazzieri S, Brambilla C, Brambilla E. Distinct pattern of E2F1 expression in human lung tumours: E2F1 is upregulated in small cell lung carcinoma. Oncogene 2001; 20:1678-79.

36. Packenham JP, Taylor JA, White CM, Anna CH, Thomaides A, Taraviras S, Tsatsas O, Zacharatos P, Karamanou E, Goumas A, et al. Expression analysis of a family of cyclin-dependent kinase inhibitor genes (p15/MTS2/p16/INK4b and p18/INK4c) in non-small cell lung cancers. Mol Carcinog 1995; 14:263-8.
expression of cyclin E in non-small cell lung cancer. Cancer Res 2000; 60:4000-4.

expression of cyclin E in resected non-small cell lung cancer. Cancer Res 2000; 60:4000-4.

expression of cyclin E in resected non-small cell lung cancer: A risk stratification model of outcome in resected non-small cell lung cancers using cyclin E. Clin Cancer Res 2000; 6:4000-4.

expression of cyclin E in resected non-small cell lung cancer stage I-IIIA. In: Lung tumors and their adrenal metastases. Lung Cancer 2007; 65:247-50.

expression of cyclin E in non-small cell lung cancer for target identification. Cancer Res 2003; 63:7185-9.

expression of cyclin E oncoprotein in resected non-small cell lung cancer. Clin Cancer Res 2006; 12:1121-7.

expression of cyclin E in non-small cell lung cancer stage I-IIIA. Clin Cancer Res 2006; 12:1121-7.

expression of cyclin E in non-small cell lung cancer. Cancer Res 2002; 62:1381-6.

expression of STK15 (Aurora-A) gene and lung cancer risk in Caucasians. Carcinogenesis 2007; 28:350-5.

expression of Wee1 is associated with an unfavourable outcome in patients with non-small-cell lung cancers. Br J Cancer 1997; 75:1774-8.

expression of Wee1 in the Chfr mitotic checkpoint gene Chfr in colorectal and non-small cell lung cancer. Carcinogenesis 2003; 24:47-51.

expression of wild-type Chfr and its relationship with clinicopathological features. Lung Cancer 2009; 66:37-47.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.

expression of cyclin E and the Chfr mitotic checkpoint gene Chfr in non-small cell lung cancer. Cancer Res 2005; 65:144-9.