TTK: A Promising Target in Malignant Tumors

Weiping Yao1#, Mingyun Jiang1#, Minjun Zhang1#, Haibo Zhang2*, Xiaodong Liang1,2*

1Graduate Department, Bengbu Medical College, Bengbu, Anhui, 23300, PR China
2Oncology Center, Department of Radiation Oncology, Zhejiang Provincial People’s Hospital, Affiliated People’s Hospital, Hangzhou Medical College, Hangzhou, Zhejiang, 310014, PR China
#These authors contribute equally to the review
*Correspondence should be addressed to Xiaodong Liang; bxdetopone@sina.com, Haibo Zhang; zhbd0ctor@163.com

Received date: July 12, 2021, Accepted date: September 15, 2021

Copyright: © 2021 Yao W, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract
TTK is a dual-specific protein kinase that phosphorylates serine and threonine. The spindle assembly checkpoint (SAC) acts as a molecular monitoring mechanism that regulates mitosis and ensures accurate separation of chromosomes. TTK is a key regulator of the SAC. Activated TTK localizes to kinetochores and activates SAC function. Simultaneously, TTK promotes error correction by phosphorylating several substrates. When all chromosomes are correctly aligned, TTK is separated from kinetochores, and SAC function is turned off, thus initiating the anaphase of mitosis. TTK is highly expressed in various malignant tumors and is negatively associated with survival. TTK inhibition inhibits malignant tumor cells growth, and the combination of TTK inhibition and chemotheraphy enhances anti-tumor effects in vitro. The combination of TTK inhibitors and chemotherapy increased efficacy without increasing adverse reactions. The combination of TTK inhibition and radiotherapy results in more DNA double-strand damage and mitotic catastrophe, with the involved mechanisms including homologous recombination pathways and non-homologous end-joining pathways. The mechanism of combination therapy is complex and requires further study. Five small-molecule TTK inhibitors have been tested in clinical trials and were used in combination with paclitaxel because the combination could enhance the killing of cancer cells and alleviate side effects by reducing the drug dosage. Because of its high expression in malignant tumors, TTK is expected to be developed as an effective anti-tumor vaccine. TTK is a promising treatment target and further investigation is warranted.

Keywords: TTK, Cancer, SAC, Inhibitor, Vaccine, Mitotic catastrophe

Introduction
TTK, also known as MPS1 (the monopolar spindle 1)/MPS1L1, is located on chromosome 6q13-q21 and encodes a dual-specific protein kinase that phosphorylates serine and threonine [1]. The spindle assembly checkpoint (SAC) plays a key role in mitosis. The SAC acts as a molecular monitoring mechanism, which delays mitosis until all chromosomes are properly attached to the spindle microtubules. As a key regulator of the SAC, TTK plays an important role in controlling cell cycle progression and maintaining genomic integrity [2]. TTK is vital for the recruitment of kinetochore components to unattached kinetochores and is essential for correcting improperly attached chromosomes. Interestingly, TTK is highly expressed in many types of malignant tumors [3]. However, TTK expression is low in most organs, except in the testis and placenta. Once TTK is inhibited, cancer cells exit mitosis prematurely, with more chromosome segregation errors and aneuploids. After several rounds of cell division, the accumulation of chromosome segregation errors may lead to cancer cell death [4]. Therefore, TTK has gradually become a research hotspot for anticancer drugs, and TTK inhibitors are increasingly being investigated in clinical trials.

The combination of TTK inhibitors and radiation therapy can increase mitotic errors, promote aneuploidy formation, inhibit DNA damage repair, and ultimately lead to mitotic catastrophe and necrosis [5]. Studies have also shown that the combination of TTK inhibitors and radiotherapy can improve the efficacy in breast cancer, liver cancer, and...
TTK is a Promising Target in Malignant Tumors

TTK is highly expressed in many types of malignant tumors. However, TTK expression is low in most organs, except in the testis and placenta [3]. In addition, high expression of TTK has been found to be associated with poor prognosis in various malignant tumors. Therefore, TTK is potentially specific antitumor target. Preclinical studies have shown that TTK inhibitors or TTK knockdown-induced mitotic aberrancies inhibit the growth of malignant tumor cells [29-31]. Moreover, TTK inhibition combined with chemotherapy has a synergistic effect in some malignant tumors [32-34]. Because of the difference in expression between malignant tumors and normal tissues, the combination of TTK inhibitors and paclitaxel increased the efficacy without increasing the adverse reactions in animal models [35].

Glioma

TTK is significantly elevated in gliomas. Overexpression of TTK was positively associated with tumor grade and negatively associated with survival in patients with glioma [32,36]. TTK knockdown resulted in mitotic aberrancies and inhibited the proliferation of glioblastoma cells. MPS1-IN-3, a selective inhibitor of TTK, caused gross chromosome segregation defects in glioblastoma cells. In addition, inhibition of TTK with MPS1-IN-3 increased the sensitivity of glioblastoma cells to vincristine both in vitro and in vivo. Moreover, the combination of MPS1-IN-3 with vincristine did not increase toxicity in animal models.

Breast cancer

Daniel et al. found that the expression levels of TTK mRNA and protein were elevated and correlated positively with aneuploidy and basal-like phenotype in breast cancer. Expression levels of TTK were positively correlated with tumor grade, p53 mutation, and poor survival. TTK knockdown caused aberrant mitosis and induced apoptosis both in vitro and in vivo. TTK inhibition tends to selectively kill cancer cells with high aneuploidy. Maire et al. reported that TTK was overexpressed in breast cancer patients, regardless of the histological type. However, the expression level of TTK was higher in triple-negative breast cancer, whereas TTK was not detected in healthy breast tissues. TTK knockdown induced mitotic catastrophe and apoptosis in triple-negative breast cancer cells. NTRC 0066-0, a selective TTK inhibitor, was developed by Maia et al. NTRC 0066-0 was found to inhibit tumor growth both in vitro and in vivo. Furthermore, simultaneous administration of NTRC 0066-0 with docetaxel extended survival and tumor remission without toxicity in a triple-negative breast cancer mouse model [33-39].

Hepatocellular carcinoma

Chemotherapy is usually ineffective in patients with advanced liver cancer with limited life expectancy. Sorafenib is often prescribed, and drug resistance soon emerges. Our previous study showed that TTK is significantly overexpressed in hepatocellular carcinoma tissues. TTK overexpression promotes hepatocellular carcinoma cell proliferation and resistance to sorafenib in vitro and in vivo. In contrast, TTK knockdown inhibits cell growth and reduces resistance to sorafenib in hepatocellular carcinoma (HCC) cells [40]. Multi-omics analysis showed that TTK mRNA levels were negatively correlated with relapse-free survival (RFS) and overall survival (OS) in patients with HCC after surgery. This indicates its potential as a prognostic biomarker [41]. It has been reported that TTK is overexpressed in 77.63% of HCC specimens, and the increased TTK expression is closely associated with tumor size and the presence of portal vein tumor thrombus. Demethylation of the TTK promoter can increase its expression in HCC. In vitro studies have shown that TTK can induce cell proliferation, colony formation and migration of HCC cells, thereby increasing the degree of malignancy of the tumor. Further studies have shown that TTK activates the Akt/mTOR pathway through p53. TTK inhibitors can inhibit the growth of HCC cells [42]. Therefore, TTK has potential therapeutic value for HCC, which encouraged us to conduct further clinical studies on TTK inhibitors in the treatment of HCC.
Pancreatic cancer

TTK is associated with poor survival of pancreatic ductal adenocarcinoma (PDAC) cells. Compared with normal pancreatic cells, PDAC is more sensitive to TTK inhibition [30]. Kaistha et al. found that TTK expression is significantly elevated in PDAC. TTK plays an important role in the growth and proliferation of PDAC cells. Loss of TTK activity can induce cell death through chromosome segregation errors. In contrast, immortalized normal pancreatic hTERT-HPNE cell lines were unaffected by TTK activity [40].

Prostate cancer

TTK inhibition has been reported to be significantly associated with the recurrence of prostate cancer, and in androgen receptor-positive prostate cancer cells, TTK inhibition enhances the antiproliferative effects of antiandrogens [43,44]. Therefore, TTK has potential clinical value in the treatment of prostate cancer.

Melanoma

Liu et al. found that in melanoma, activated B-Raf (V600E) prevented TTK degradation and increased the amount of TTK protein, which led to centrosome amplification and incorrect chromosomal segregation [45]. B-Raf/MEK/ERK signaling and Mps1/Akt constitute an automatically regulated negative feedback loop in melanoma cells. Oncogenic B-RAF (V600E) can eliminate the negative feedback loop and cause TTK dysfunction to induce chromosome instability and tumorigenesis [46]. Therefore, it has been suggested that combined targeted therapy of B-RAF and TTK should be applied in clinical practice.

Lung cancer

Suda et al. established a strong cytotoxic T lymphocytes (CTL) clone stimulated by TTK-567 with specific killing activity against HLA-A24-positive lung cancer and esophageal cancer cells [47]. This feature illustrates the potential of TTK as a cancer vaccine. TTK peptide vaccine has also been shown to be safe and well-tolerated in late-stage clinical trials in patients with advanced or recurrent non-small cell lung cancer (NSCLC) [48].

Esophageal cancer

Mizukami et al. found that in patients with esophageal squamous cell carcinoma (ESCC), TTK antigen can induce a specific T cell response [49]. In clinical trials, the efficacy and safety of the TTK vaccine for advanced esophageal cancer have been confirmed [50]. This will encourage us to further study the use of a TTK tumor vaccine in advanced ESCC.

Biliary tract cancer

In clinical trials, the TTK peptide vaccine induced specific T cell immune responses in patients with advanced biliary tract cancer (BTC) and achieved good efficacy. This will lead us to regard TTK vaccination therapy as one of the few treatment options for advanced BTC [51].

TTK Inhibitors

Researchers have used small molecule compounds to block the function of the SAC by inhibiting TTK activity, resulting in the disruption of mitotic stability. This feature can be exploited to develop cancer treatment strategies. To date, several types of small-molecule compounds that inhibit TTK activity have been identified or developed. These small molecule compounds can be divided into four broad groups, including N-phenylpyrimidin-2-amine, N-phenylpyridine, heterocyclic small compounds in 3-phenylindazole, and 5-membered bridged six-membered bridged [52] (Table 1). Based on the mechanism of action of TTK inhibitors, researchers used TTK inhibitors in combination with microtubule-targeting agents (MTAs) to increase chromosomal separation errors and kill cancer cells more efficiently. Some studies have shown that TTK inhibitors combined with chemotherapeutic agents can effectively increase the killing effect of tumor cells. Wu et al. found that CC-671 (a highly selective inhibitor of TTK and CLK2) could inhibit the drug efflux activity of ABCG2 in lung cancer cells, thus increasing the level of intracellular chemotherapy drugs. This will help improve the efficacy of chemotherapy in lung cancer [34]. Tannous et al. developed

| Table 1: TTK Inhibitors Classified by Chemical Structure. |
|-------------------|-----------------------------|
| Chemical structure                                      | Drug name                        |
| 1. Compounds with N-phenylpyrimidin-2-amine scaffolds     | Reversine, MPI-0479605, Mps1-IN-3, AZ 3146, CC-671, BOS-172722, Mps1-IN-2, NTRC 0066-0, NMS-P715 et al. |
| 2. Compounds with N-phenylpyridine scaffolds              | TC Mps112, Mps1-IN-1, CCT251455 et al. |
| 3. Compounds with 3-phenylindazole scaffold             | SP600125, CFI-400936, CFI-401870 et al. |
| 4. Compounds with five-membered bridged six-membered heterocyclic scaffolds | Mps-BAY1, Mps-BAY2a, Mps-BAY2b, BAY 1161909, BAY 1217389, CFI-402257, PF-7006, PF-3837 et al. |
a selective small molecule inhibitor of TTK, Mps1-IN-3, which caused mitotic abnormalities in glioblastoma cells, and the combination of Mps1-IN-3 and vincristine increased aneuploidy and cell death. In addition, Mps1-IN-3 increases the susceptibility of glioblastoma cells to mitotic drugs [32]. Similarly, inhibition of TTK enhanced the efficacy of docetaxel in a triple-negative breast cancer model [33].

**TTK Inhibitors in Clinical Trials**

There are currently five small-molecule TTK inhibitors in clinical trials, namely BAY-1217389 [35,53,54], BAY-1161909 [35,53,54], BOS-172722 [55,56], CFI-402257 [2,57-60], and S-81694 (Table 2). Among them, BAY-1217389, BOS-172722, and S-81694 completed phase I or II trials. The CFI-402257-related clinical trial is still in its recruitment phase. However, clinical trials on BAY-1161909 have been terminated. All five inhibitors were used in combination with paclitaxel, because the combination not only increased the sensitivity of cancer cells, but also reduced side effects by reducing the dose of the drug. It is worth noting that determining the tolerance and toxicity due to long-term use of TTK inhibitors requires further study [61,62]. When cancer cells are resistant to one TTK inhibitor, they can be combined with several other sensitive TTK inhibitors to solve the problem of resistance. TTK inhibitors have unique advantages for tumor therapy. TTK inhibitors can target excessive TTK in tumors with little effect on normal cells, and can improve the sensitivity of cancer cells to paclitaxel and other chemotherapy drugs. Therefore, TTK inhibitors can be used in combination with existing anti-tumor drugs or treatment regimens to enhance tumor killing and improve survival in cancer patients.

**Inhibition of TTK Improve Radiosensitivity in Malignant Tumors**

Radiotherapy is an important treatment method for malignant tumors. Both TTK and radiotherapy affect mitosis and DNA damage repair, and the combination of TTK inhibition and radiotherapy may have a synergistic effect. Some preclinical studies have investigated such effects and their mechanisms in malignant tumor models (Table 3).

---

**Table 2: TTK Inhibitors in Clinical Trials.**

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Study Title</th>
<th>Disease</th>
<th>Intervention</th>
<th>Phase</th>
<th>NCT Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY-1217389 (Completed)</td>
<td>Phase I Study of Oral BAY-1217389 in Combination With Intravenous Paclitaxel</td>
<td>Advanced malignancies (solid tumors)</td>
<td>BAY-1217389 + paclitaxel</td>
<td>I</td>
<td>NCT02156949</td>
</tr>
<tr>
<td>BAY-1161909 (Terminated)</td>
<td>Phase I Dose Escalation of Oral BAY-1161909 in Combination With Intravenous Paclitaxel</td>
<td>Advanced malignancies (solid tumors)</td>
<td>BAY-1161909 + paclitaxel</td>
<td>I</td>
<td>NCT02138812</td>
</tr>
<tr>
<td>BOS-172722 (Completed)</td>
<td>Study of Paclitaxel in Combination With BOS-172722 in Patients With Advanced Nonhaematologic Malignancies</td>
<td>Advanced Nonhaematologic Malignancies (ANM)</td>
<td>BOS-172722 + paclitaxel</td>
<td>I</td>
<td>NCT0328494</td>
</tr>
<tr>
<td>CFI-402257 (Recruiting)</td>
<td>A Study of Investigational Drug CFI-402257 in Patients With Advanced Solid Tumors</td>
<td>Advanced Solid Cancers (ASC), Breast Cancer</td>
<td>CFI-402257 + Fulvestrant</td>
<td>I</td>
<td>NCT02792465</td>
</tr>
<tr>
<td></td>
<td>CFI-402257 in Combination With Paclitaxel in Patients With Advanced/Metastatic HER2-Negative Breast Cancer</td>
<td>Breast Cancer</td>
<td>CFI-402257 + paclitaxel</td>
<td>II</td>
<td>NCT03568422</td>
</tr>
<tr>
<td>S-81694 (Completed)</td>
<td>S-81694 Plus Paclitaxel in Metastatic Breast Cancer</td>
<td>Metastatic Breast Cancer (mBC)</td>
<td>S-81694 + paclitaxel</td>
<td>I</td>
<td>NCT03411161</td>
</tr>
</tbody>
</table>

[https://www.clinicaltrials.gov/](https://www.clinicaltrials.gov/)
Gliomas are most widely reported in this field. Maachani et al. found that a TTK inhibitor, NMS-P715, increased the radiosensitivity of glioblastoma (GBM) cells by reducing DNA double-strand break repair and inducing mitotic catastrophe after radiation. The involved mechanisms included both homologous recombination and non-homologous end-joining pathways in that study [7]. Further studies showed that TTK inhibition regulates the tumor suppressors PDCD4 and MSH2 through miR-21. MiR-21 has been shown to be elevated after radiation and mediates the radiation resistance of glioblastoma cells by regulating PDCD4 and MSH2 [63]. Another study found that hepatic leukemic factor (HLF) can inhibit the expression of TTK through miR-132, thereby inhibiting the proliferation, metastasis, and radiation resistance in GBM cells [64].

The combination of TTK and radiotherapy has also attracted attention in other cancers. Our recent study showed that the inhibition of TTK blocked G2/M transition in the cell cycle by upregulating p21 and enhancing radiosensitivity in liver cancer cells [8]. We also found that the combination of TTK inhibition and radiotherapy enhanced mitotic catastrophe and DNA damage. This opens up a new field for the treatment of liver cancer. Interestingly, low-dose (non-cytotoxic) TTK inhibitors showed radiosensitization in this study. The combination of low-dose TTK inhibitors and radiotherapy is expected to improve tumor control without increasing adverse reactions, which warrants further study. In addition, TTK may be responsible for radiation resistance in patients with basal breast cancer. Chandler et al. found that radiosensitivity was enhanced by the inhibition of TTK both in vitro and in vivo in basal-like breast cancer cells. TTK inhibition leads to unrepairred double-stranded DNA damage by reducing the repair efficiency of the homologous recombination system. However, nonhomologous end-joining was not considered in this study [6,65].

**Conclusions**

TTK is the key regulator of the SAC, which regulates mitosis and ensures the accurate separation of chromosomes. TTK is highly expressed in various malignant tumors, while its expression is low in most normal organs. TTK is positively associated with grade and associated negatively with survival in various malignant tumors. Preclinical studies show that TTK inhibition induces mitotic aberrancies and high aneuploidy and inhibits the growth of malignant tumor cells. The combination of TTK inhibitors and chemotherapy increases efficacy without increasing adverse reactions in animal models. The addition of TTK inhibition also enhanced the effects of radiotherapy. The combination of TTK inhibition and radiotherapy results in more DNA double-strand damage and mitotic catastrophe. Involved mechanisms may include homologous recombination, non-homologous end-joining pathways, or both. The mechanism of combination therapy has not yet been clarified. Several types of small-molecule TTK inhibitors have been developed. Five small-molecule TTK inhibitors have been tested in clinical trials. All five inhibitors were used in combination with paclitaxel because the combination may not only enhance the killing of cancer cells, but also alleviate side effects by reducing the dose of paclitaxel (Figure 1). Because of its high specific expression in malignant tumors, TTK is expected to be developed as an effective anti-tumor vaccine. The efficacy and safety of the TTK vaccine have been tested in advanced esophageal, biliary tract, and lung cancer. In conclusion, targeting TTK has broad prospects, not only is the TTK inhibitor expected to be combined with radiotherapy and chemotherapy safely to further improve the curative effect, but the TTK vaccine is expected to be effective in the treatment of malignant tumors. However, the biomarkers for predicting the efficacy of TTK inhibitors are still lacking, the specific molecular mechanism of TTK inhibitors needs to be further studied, and the drug resistance mechanism...
of TTK inhibitors is not well understood. The sequence of radiotherapy and chemotherapy combined with TTK inhibitors and the adjustment of dose also need to be further studied.

Declaration of Competing Interest

The authors have no interest to declare.

Acknowledgments

This study was supported in part by grants from the Zhejiang Province Medical and Health Care Project (2019KY279 to Xiaodong Liang) and the National Natural Science Foundation of China (Grant number:82003236 to Haibo Zhang).

References


