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Commentary

A new screening method for ATP-independent kinase inhibitors identifies repurposed anti-cancer drugs☆

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In the era of targeted drugs for cancer, small molecule kinase inhibitors have been designed to target specific downstream molecules in tumor-promoting signaling pathways; however, such compounds often exhibit high toxicity profiles due in part to their off-target effects [1]. This limits the drug dosage that can be used safely, and concomitantly, decreases drug efficacy. Therefore, there is a clear need to identify inhibitory molecules with high affinity and greater specificity against their target.

In this issue of EBioMedicine, Cheng et al., describe a novel and highly efficient drug screening technology for the identification of potent and specific molecules that inhibit Dbf4-dependent kinase (DDK) [10]. DDK as well as cyclin dependent kinases (CDKs) are proteins associated with cell cycle. As such, they have been considered as major targets for drug screening [2]. Among these proteins, cell division cycle 7-related protein kinase (Cdc7) is a serine threonine kinase with an important role in initiating DNA replication and cell cycle control in eukaryotic cells. Cdc7 kinase activity is regulated by Dbf4 activation unit and in turn promotes the initiation of DNA synthesis by phosphorylation of MCM2–7 helicase complex throughout the S phase [3]. Indeed, in line with its crucial function, Cdc7 was shown to be overexpressed in multiple malignant conditions, including breast, colon, lung, ovarian carcinomas as well as diffuse large B cell lymphoma, and oral squamous cell carcinoma (OSCC) [4]. Besides DNA replication initiation, Cdc7/Dbf4 kinase maintains DNA damage tolerance and increases cancer cell survival [3]. Noteworthy, a strong correlation between the Cdc7/Dbf4 kinase expression levels and mutational burden of the tumor was recently demonstrated, suggesting that increased DDK levels promote mutagenesis and contribute to chemoresistance [5]. Therefore, Cdc7 inhibitors can potentially serve as a useful therapy for cancer. Indeed, in recent years, a large number of Cdc7 inhibitors have been developed to blunt pro-tumorigenic activity. For instance, XL413, a benzofuropyrimidinone selective ATP-competitive Cdc7 inhibitor was shown to reduce viability and proliferation of OSCC cells and enhance the cytotoxic effect of cisplatin and 5-fluorouracil [4]. However, like the majority of small molecule kinase inhibitors that attenuate Cdc7 activity, XL413 is an ATP-competitive inhibitor, namely, it blocks the ATP-binding site of the kinase. As such, it may also interfere with the activity of other essential kinases. These off-target effects lead to decreased efficacy due to dose-limitation. Therefore, there is great value in developing agents that can inhibit kinase activity in an ATP-independent manner.

The method developed by Cheng et al., [10] is based on a protein-fragment complementation assay with a *Renilla reniformis* luciferase readout (Rluc-PCA). It allows accurate quantification of protein-protein interactions and instant protein activity in live cells due to its sensitivity, high signal to background ratio and reversibility. Importantly, rather than searching for molecules that compete for ATP binding, the method is specifically designed to identify molecules that interfere with the interaction between Dbf4 and Cdc7. Using this technology, the authors performed a high-throughput screen of FDA-approved compounds in live OSCC cells. Their screen revealed two compounds, dequalinium chloride and clofoctol, that specifically inhibited DDK activity in cancer cells and attenuated oral carcinoma tumor growth in vitro and in a preclinical mouse model.

Clofoctol and dequalinium compounds are widely used agents due to their profound antimicrobial effects. However, in addition to their initial purpose, a growing body of evidence suggests that they also act as anti-cancer drugs. For example, clofoctol that kills bacteria by disrupting membrane permeability, was recently reported to attenuate prostate cancer cell growth by indirectly inhibiting protein translation and subsequently inducing cell cycle arrest [6]. Similarly, various dequalinium analogues were shown to possess anti-cancer activity by selectively accumulating in mitochondria of cancer cells thus interfering with cell energy, and also by specifically targeting cancer stem cells [7,8].The discovery that these molecules disrupt the protein-protein interaction between Dbf4 and Csc7 [10] provides a new possible molecular explanation for their anti-tumor activity.

Lastly, Cheng et al. describe synergistic effects when dequalinium chloride therapy is combined with chemotherapy and radiotherapy. They claim that these newly identified DDK inhibitors sensitize tumors to treatment-induced DNA damage such as radiotherapy or platinum-based chemotherapy, therefore increasing the cytotoxic effects of the latter. Their observation is in line with previous reports demonstrating that other dequalinium analogs e.g., dequalinium-14, combined with radiotherapy reduce metastasis in mice bearing colorectal carcinoma [9].

☆ Commentary on the manuscript "Identification of novel Cdc7 kinase inhibitors as anti-cancer agents that target the interaction with Dbf4 by the fragment complementation and drug repositioning approach"

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To summarize, Cheng et al. [10] introduce an efficient and elegant method to discover novel therapeutic agents that block pro-tumorigenic targets in the intracellular machinery. Since their method specifically focuses on inhibiting protein-protein interactions in an ATP-independent manner, it allows for the discovery of highly specific drugs with greater potential to overcome the major limitation of off-target toxicity.

Conflict of interest

The authors declare no conflict of interest.

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