MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 6, e1673643 (3 pages)
https://doi.org/10.1080/23723556.2019.1673643

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AUTHOR'S VIEW

CDK4/6 inhibition in cancer: the cell cycle splicing connection

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ABSTRACT

Cyclin-dependent kinase −4 and −6 (CDK4/6) inhibitors are currently being assessed in clinical trials for the treatment of many cancers including melanoma. While investigating the mechanisms of CDK4/6 inhibitor resistance in melanoma, we uncovered a mechanism of action of these inhibitors in regulating the expression of both the mouse double minute 4 (MDM4) oncogene and tumor protein p53 (TP53).

ARTICLE HISTORY
Received 19 September 2019
Revised 22 September 2019
Accepted 24 September 2019

KEYWORDS
CDK4; CDK6; melanoma; acquired resistance; PRMT5; MDM4; TP53; p21; pre-mRNA splicing

Cyclin-dependent kinase −4 and −6 (CDK4/6) are serine/threonine kinases that when complexed with Cyclin D1 are central regulators of the G1-S transition of the cell cycle. CDK4/6 promote cell cycle progression by phosphorylation and inhibition of the tumor suppressor, retinoblastoma protein (RB1). They also phosphorylate many other targets,[1] of which the functional consequences are largely unknown. The hyperactivation of CDK4/6 observed in most cancers, together with the key role they play in regulating the cell cycle, has spearheaded the development of CDK4/6 small molecule inhibitors. To date three inhibitors namely, palbociclib, ribociclib, and abemaciclib are approved for clinical use in combination with endocrine therapy in breast cancer. The excitement that surrounds the potential use of these inhibitors for the treatment of cancer is demonstrated by the number (over 180) of completed or ongoing clinical trials testing CDK4/6 inhibitors across a broad portfolio of cancers (https://clinicaltrials.gov/).

With all cancer therapies, the development of resistance is a major obstacle, thus identifying the mechanisms of response and resistance to these therapies is vital in developing rational combination strategies to improve antitumor efficacy. Primary resistance to CDK4/6 inhibitors is predominantly due to loss of functional RB1, while preclinical studies have demonstrated several mechanisms of acquired resistance including hyperactivation of the CDK2-cyclin E complex.[2] In CDK2 mediated resistance, CDK2 substitutes for CDK4/6 by phosphorylating RB1 and thus maintaining cell cycle progression.

In order to identify mechanisms of acquired resistance to palbociclib in melanoma, we employed multiple high throughput approaches including reverse phase protein arrays, global gene expression analysis and a drug screen.[3] What we uncovered was that in melanoma cells, CDK4/6 inhibitors ultimately led to CDK2 inhibition via a novel signaling pathway (Figure 1). Specifically, in drug sensitivity cells, palbociclib suppressed protein arginine methyltransferase 5 (PRMT5) activity, a known modulator of pre-mRNA splicing, which resulted in alterations in mouse double minute 4 (MDM4) pre-mRNA splicing and reduced expression of MDM4 protein. In tumor protein p53 (TP53)-wildtype cells, loss of MDM4 led to TP53 activation and induction of cyclin-dependent kinase inhibitor 1A (p21), a known inhibitor of CDK2 activity. In acquired-resistance cells, palbociclib failed to decrease PRMT5 activity, and consequently failed to decrease MDM4 expression and activate TP53. Treatment of TP53-wildtype palbociclib-resistant cell lines with a combination of a PRMT5 inhibitor and palbociclib decreased RB1 phosphorylation and cell proliferation similar to the response observed with a combination of palbociclib and a CDK2 inhibitor. This data indicated that decreasing PRMT5 activity led to decreased CDK2 activity, likely via inducing the CDK2 inhibitor p21.

While the ability of CDK2 to substitute for CDK4/6 activity in CDK4/6 inhibitor resistant cells is not a novel finding, palbociclib regulation of PRMT5-MDM4-TP53-p21 axis leading to CDK2 activation is. Furthermore, our studies have shown a role of CDK4/6 inhibitors in modulating pre-mRNA splicing. RNA splicing results in a multitude of transcripts that contribute to the cells proteome diversity. Aberrant splicing plays a role in cancer and is also associated with epithelial-to-mesenchymal transition and cell "stemness",[4,5] two processes important in cancer therapy resistance. Understanding the complement of mRNA splice variants induced by CDK4/6 inhibitors will add insight into the mechanism of action of these therapeutics and potentially expand their clinical application.

Although palbociclib can modulate PRMT5 activity, robust inhibition of PRMT5 activity with a small molecule inhibitor together with palbociclib induced strong inhibition of cell proliferation across several cancer types, including pancreatic, esophageal and breast cancer. All the cell lines had an activated CDK4/6 pathway and expressed high levels of MDM4. Interestingly, the panel included four TP53-mutant cell lines, all of which responded to palbociclib as a single agent, but the response was far greater when combined with a PRMT5 inhibitor. This data showed that these inhibitors, in addition to being able to activate TP53-wildtype, also worked efficiently in a TP53-independent manner.

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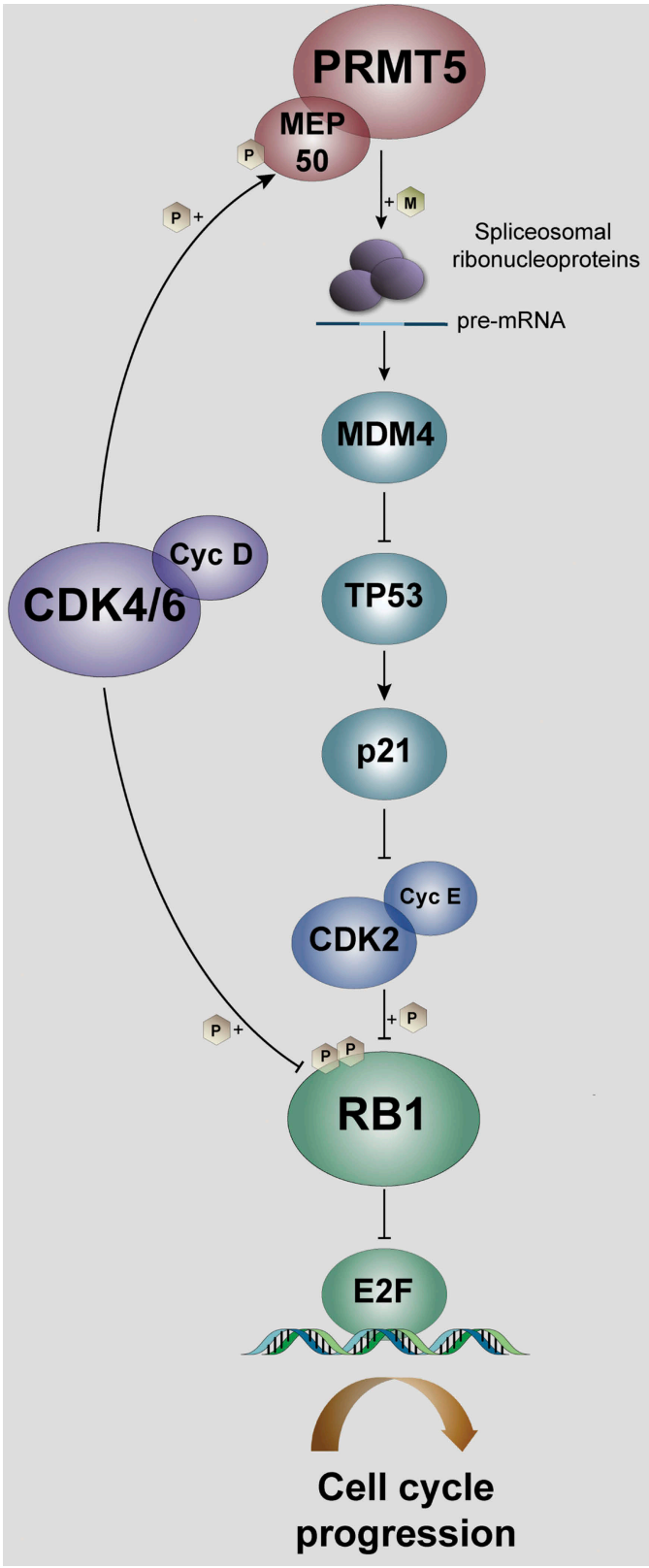

Figure 1. CDK4/6-PRMT5-RB1 axis. Cyclin-dependent kinase −4 and −6 (CDK4/6)-cyclin D1 complex phosphorylates retinoblastoma protein (RB1) and promotes cell cycle progression, in addition this complex phosphorylates many other proteins including methylosome protein 50 (MEP50), a critical co-activator of protein arginine methyltransferase 5 (PRMT5). Once activated, PRMT5 methylate's an array of proteins, most importantly proteins that are essential components of the spliceosome machinery. Methylation of spliceosomal ribonucleoproteins by PRMT5 results in altered splicing of mouse double minute 4 (MDM4) pre-mRNA and the production of a functional isoform. Increased MDM4 expression supresses tumor protein p53 (TP53) activity, leading to decreased expression of the CDK2-cyclin E complex inhibitor cyclin-dependent kinase inhibitor 1A (p21), subsequent increased CDK2 activity and phosphorylation of RB1 which results in E2F mediated gene transcription.

Our studies set out to understand the mechanism of resistance to palbociclib in melanoma and what we uncovered was a link between CDK4/6 activity and MDM4 expression via PRMT5. PRMT5 through altering RNA splicing and methylating many cellular proteins impacts on a multitude of cellular functions[6] and MDM4 has a range of functions both dependent and independent of TP53.[7] Thus, CDK4/6 regulation of both PRMT5 and MDM4 likely extends the mechanism of action of CDK4/6 inhibitors far beyond regulation of the cell cycle.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Peter MacCallum Cancer Foundation (AU); Cancer Council Victoria (AU) Grant under Grant [1108149]; and National Health and Medical Research Council of Australia under Grant [1042986].

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