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CCRK is a novel signalling hub exploitable in cancer immunotherapy

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ABSTRACT

Cyclin-dependent kinase 20 (CDK20), or more commonly referred to as cell cycle-related kinase (CCRK), is the latest member of CDK family with strong linkage to human cancers. Accumulating studies have reported the consistent overexpression of CCRK in cancers arising from brain, colon, liver, lung and ovary. Such aberrant up-regulation of CCRK is clinically significant as it correlates with tumor staging, shorter patient survival and poor prognosis. Intriguingly, the signalling molecules perturbed by CCRK are divergent and cancer-specific, including the cell cycle regulators CDK2, cyclin D1, cyclin E and RB in glioblastoma, ovarian carcinoma and colorectal cancer, and KEAP1-NRF2 cytoprotective pathway in lung cancer. In hepatocellular carcinoma (HCC), CCRK mediates virus-host interaction to promote hepatitis B virus-associated tumorigenesis. Further mechanistic analyses reveal that CCRK orchestrates a self-reinforcing circuitry comprising of AR, GSK3β, β-catenin, AKT, EZH2, and NF-κB signalling for transcriptional and epigenetic regulation of oncogenes and tumor suppressor genes. Notably, EZH2 and NF-κB in this circuit have been recently shown to induce IL-6 production to facilitate tumor immune evasion. Concordantly, in a hepatoma preclinical model, ablation of *Ccrk* disrupts the immunosuppressive tumor microenvironment and enhances the therapeutic efficacy of immune checkpoint blockade *via* potentiation of anti-tumor T cell responses. In this review, we summarized the multifaceted tumor-intrinsic and -extrinsic functions of CCRK, which represents a novel signalling hub exploitable in cancer immunotherapy.

Contents

Abbreviations: 9G8, serine and arginine rich splicing factor 7; AKT, AKT serine/threonine kinase; AR, androgen receptor; ARE, androgen response element; ARG1, arginase 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and rad3-related protein; BMDC, mouse bone marrow-derived dendritic cell; CAK, CDK-activating kinase; CaMKV, CaM kinase like vesicle associated; Cas9, CRISPR-associated protein-9 nuclease; CBP, CREB binding protein; CCND1, cyclin D1; CCRK, cell cycle-related kinase; CDK, cyclin-dependent kinase; C/EBPβ, CCAAT-enhancer-binding protein; CHK1, checkpoint kinase 1; CHK2, checkpoint kinase 2; CRISPR, clustered regularly interspaced short palindromic repeats; DNMT1, DNA methyltransferase 1; DSIF, DRB sensitivity inducing factor; E2F1, E2F transcription factor 1; EGFR, epidermal growth factor receptor; ER, estrogen receptor; EZH2, enhancer of zeste homolog 2; FDA, Food and Drug Administration; FOXM1, forkhead box M1; FOXO1, forkhead box O1; FOXP3, forkhead box P3; GCLM, glutamate-cysteine ligase modifier subunit; GDC, genomic data commons; GLI, glioma-associated oncogene homolog; GSK3β, glycogen synthase kinase 3 beta; H3K27, histone H3 lysine 27; H3R8, histone H3 arginine 8; H4R3, histone H4 arginine 3; HAT, histone acetyltransferase; HBV, hepatitis B virus; HBx, hepatitis B virus X protein; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; Hh, hedgehog; HIV-1, human immunodeficiency virus-1; HR, hormone receptor; ICK, intestinal cell kinase; IFN-γ, interferon gamma; IFT, intraflagellar transport; IL-2, interleukin 2; IL-6, interleukin 6; IL-12, interleukin 12; IRF2, interferon regulatory factor 2; IRF2BP2, interferon regulatory factor 2 binding protein 2; KEAP1, kelch like ech associated protein 1; MAK, male germ cell associated kinase; MAPK, mitogen-activated protein kinase; MAT1, MNAT CDK-activating kinase assembly factor 1; MAX, MYC associated factor X; MDSC, myeloid-derived suppressor cell; MST3, serine/threonine kinase 24; MYC, myc proto-oncogene protein; MYCN, *N*-myc proto-oncogene protein; mTOR, mechanistic target of rapamycin; NELF, negative elongation factor complex member E; NF-κB, nuclear factor kappa B; NQO1, NAD(P)H quinone dehydrogenase 1; NRF2, NF-E2-related factor 2; p-TEFb, positive transcription elongation factor b; p50, nuclear factor NF-kappa-B p50 subunit; p65, nuclear factor NF-kappa-B p65 subunit; p300, E1A binding protein; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinases; PMN, polymorphonuclear; PRDX1, peroxiredoxin 1; PRMT5, protein arginine methyltransferase 5; PTCH1, patched 1; RB, retinoblastoma protein; RNAPII, RNA polymerase II; RUNX1, runt related transcription factor 1; SC35, serine and arginine rich splicing factor 2; SMO, smoothened, frizzled class receptor; STAT3, signal transducer and activator of transcription 3; SUFU, SUFU negative regulator of hedgehog signalling; SUMO2, small ubiquitin-like modifier 2; TCF, transcription factor; TFIIH, transcription factor II human; TDRD7, tudor domain containing 7; Treg, regulatory T cell; WDR77, WD repeat domain 77; WNT, WNT family member.

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1. Introduction

Cyclin-dependent kinases (CDKs) are a family of enzymes that play critical roles in eukaryotic cells. To date 21 CDKs (including CDKs 1–10, 11A–B, and 12–20) have been identified in human, which can be sub-divided into 11 groups according to their evolutionary relationships and sequence similarities (Malumbres et al., 2009). Regardless of their different structures and substrate specificities, each CDK possesses a conserved catalytic domain for kinase activity, a domain for cyclin binding, and a T-loop motif as a functional switch (Lolli, 2010). Previous studies have clearly established the specific and redundant roles of CDKs in cell cycle control. Intriguingly, emerging evidence shows that CDKs can also take part in many other cellular activities, including but not limited to transcriptional regulation, DNA repair and cytoskeleton dynamics.

1.1. Cell cycle-dependent roles of CDKs

In eukaryotes, cell cycle is a sophisticated and tightly controlled process, which consists of five different phases: G_0 (quiescence), G_1 (preparation), S (DNA synthesis), G_2 (checkpoint), and M (cell division). These ordered events are fundamentally governed by multiple CDKs and cyclins (Pines, 1994) (Fig. 1). When a cell is presented with growth factors, cell surface receptors and signalling pathways are activated (*e.g.* RAS, MAPK, mTOR) (Malumbres & Barbacid, 2001; Rodgers et al., 2014), and mitogenic signals are transmitted intracellularly to drive G_0-to-G_1 progression. Following G_1 entry, the structurally related CDK4 and CDK6 are bound and activated by D-type cyclins (including D1, D2 and D3) (Sherr, 1995), which then phosphorylate the tumor suppressor RB, the related proteins RBL1 and RBL2, and also the transcription factor FOXM1 (Anders et al., 2011; Sherr, 1995). Such phosphorylation is a step critical for disrupting the binding and suppression effects of RB (and other co-repressors) on E2F transcription factors; as a consequence the transcriptional activities of E2F are resumed, initiating the expression of a multitude of molecules, such as cyclin A, cyclin E, CDK1 and CDK2, that are required for G_1-to-S progression (Adams, 2001; Burkhart & Sage, 2008). At this stage, cyclin A and cyclin E bind to and activate CDK2, which further phosphorylates RB in a positive feedback loop, and facilitates the process of DNA synthesis in S phase (Mittnacht, 1998). When DNA replication is completed in G2 phase, CDK1 is bound and activated by cyclin A and B to phosphorylate additional targets promoting chromosomal segregation, cytokinesis, and finally separation of two daughter cells (Datta et al., 1996; Wheatley et al., 1997).

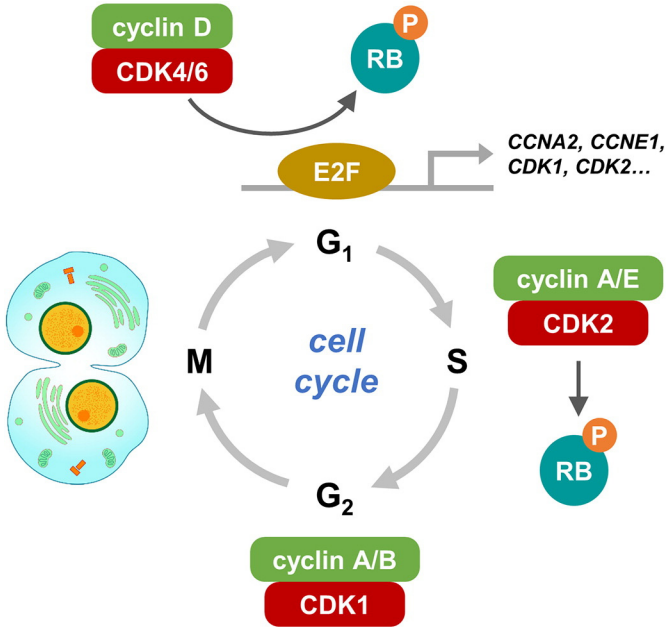

Fig. 1. CDKs and cyclins in cell cycle. Schematic diagram showing the functions of different CDK-cyclin pairs in individual cell cycle phases. In G_1 phase, CDKs 4 or 6 coupled with cyclin D phosphorylates and removes RB from E2F to facilitate the transcription of cell cycle effectors *e.g. CCNA2*, *CCNE1*, *CDK1*, etc. In S phase, CDK2 coupled with cyclin A or E reinforces the phosphorylation of RB to promote DNA synthesis. In G_2 phase, CDK1 coupled with cyclin A or B phosphorylates additional targets for progression into mitosis.

1.2. Cell cycle-independent roles of CDKs

Apart from cell cycle control, gene transcription is another process coordinated by CDKs. For instance, CDK7 is a part of the transcription factor II human (TFIIH) complex, wherein CDK7, MAT1 and cyclin H form a CDK-activating kinase (CAK) subunit that can phosphorylate the serine amino acids of the C-terminal domain of RNA polymerase II (RNAPII) (Serizawa et al., 1995; Shiekhattar et al., 1995), leading to gene transcription, recruitment of the integrator complex and expression of small nuclear RNAs (Egloff et al., 2007, 2010; Glover-Cutter et al., 2009). Moreover, CDK9 and cyclin T1 form a subunit of the positive transcription elongation factor b (P-TEFb) complex, which can also phosphorylate RNAPII, and is necessary for transcription elongation and 3′ end RNA processing (Larochelle et al., 2012).

Besides transcription, CDKs are also involved in various DNA damage checkpoints. In the presence of genotoxic insults, induced or spontaneous DNA lesions are promptly sensed by ATM and ATR, leading to phosphorylation and activation of CHK1, CHK2 and p53, which can respectively arrest cell cycle at G1/S/G2 phase through inhibition of CDK1/2 (Shiloh, 2003). CDK5 also responds to DNA damage by binding to and phosphorylating STAT3, thereby modulating the binding and activation effects of STAT3 on specific target genes such as endonuclease *EME1* to promote DNA repair (Courapied et al., 2010).

In the context of the cytoskeleton, CDK5 is crucial in the regulation of neurite outgrowth, neuronal migration, axon guidance, and synapse formation. Previous studies have shown that CDK5 controls actin dynamics in neuronal tissues by phosphorylating a range of substrate proteins, for examples, ephexin1, CaMKV, MST3 and p27 (Shah & Rossie, in press). In addition, CDK18 was recently found to suppress cell migration by inhibiting the RhoA/ROCK signalling pathway (Matsuda, Kawamoto, Miyamoto, Tsuji, & Yuasa, 2017). Altogether, these examples demonstrate the functional diversity of CDKs in eukaryotic cells.

1.3. Knowledge gaps in CDKs

The research interests on individual CDKs varied significantly over the past decades. In general, CDK1–2 and CDK4–9 are the most extensively studied CDKs, with hundreds to over a thousand reports in PudMed. This is probably due to their apparent roles in cell cycle and

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