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The role of FZR1 in tumorigenesis: Focus on cell-cycle control

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Abstract: Fizzy-related protein homolog 1 (FZR1) mainly functions as a specific activator of the anaphase-promoting complex/cyclosome (APC/C) in the cell cycle and controls the G0 and G1 phases of the cell cycle. We highlight recent work that has studied the role of FZR1 in tumorigenesis, growth, differentiation, and genome stability through cell-cycle control. We summarize the current state of knowledge regarding FZR1 structure, function, and the distinct ways of APC/C dysregulation in solid tumors and hematologic malignancies. We also discuss novel approaches for targeting the FZR1 as a cancer therapy and research area for future work.

Abbreviations

APC/C	The anaphase-promoting complex/cyclosome
AMPK	AMP-activated protein kinase
Cdh1	Cell division cycle 20 related 1
CDC20	Cell division cycle 20 homolog
CDK	Cyclin-dependent kinase
CENP-T	Centromere protein T
CSC	Cancer stem-like cells
CPC	Chromosomal passenger complex
ECs	Endothelial cells
FZR1	Fizzy-related protein homolog 1
HSCs	Hematopoietic stem cell
MM	Multiple myeloma
MEKK2	Mitogen-activated protein kinase 2
PLK1	Polo-like kinase 1
pRb	Retinoblastoma protein
SAC	Spindle assembly checkpoint
SKP2	S-phase kinase-associated protein 2
topiα	Telomerase IIα
SCs	Satellite cells
SPOP	Speckle-type POZ protein
TBK1	Tank binding kinase 1

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Introduction

Fizzy-related protein homolog1 (FZR1) is named after the Drosophila homolog fizzy, the anaphase-promoting complex/cyclosome (APC/C) coactivator, and is therefore also called CDC20 homolog1 (Cdh1). FZR1 mainly comprises a β-propeller with a seven-leaf WD40 repeat and a C-box containing important functional motifs (Pines, 2011; Primorac and Musacchio, 2013) (Figs. 1A and 1B). It can use its WD40 repeat domain to recruit to different APC/C (the anaphase-promoting complex/cyclosome) substrates for ubiquitination degradation of the substrates. The D box directly interacts with the evolutionarily conserved surface on the WD40 propeller structure of FZR1. It is activated by the binding of CDC27, allowing APC/C to recognize the substrates D box and KEN box (Harper et al., 2002; Kraft et al., 2005; Brown et al., 2015).

The ligand for FZR1 Is APC/C, a conserved multi-subunit E3 ubiquitination ligase consisting of 14 proteins with 19 subunits and 3 associated adaptors. It contains a catalytic core, platform, and tetrapeptide repetition, including APC1~APC16, and is primarily known for its ability to trigger a mid-to-late transition in mitosis. Its crucial role in cell division makes it essential for survival and development, and is involved in abnormal regulation of tumorigenesis (Peters, 2006; Thornton and Toczyski, 2006; Wang et al., 2009; Schreiber et al., 2011; Uzunova et al., 2012; Chang et al., 2015; Yamaguchi et al., 2015). Ubiquitination of substrates by APC/C requires the formation of an APC/C-activator-substrate complex. FZR1 and CDC20 act as activators of APC/C to induce conformational changes in the active forms APC/C^CDC20
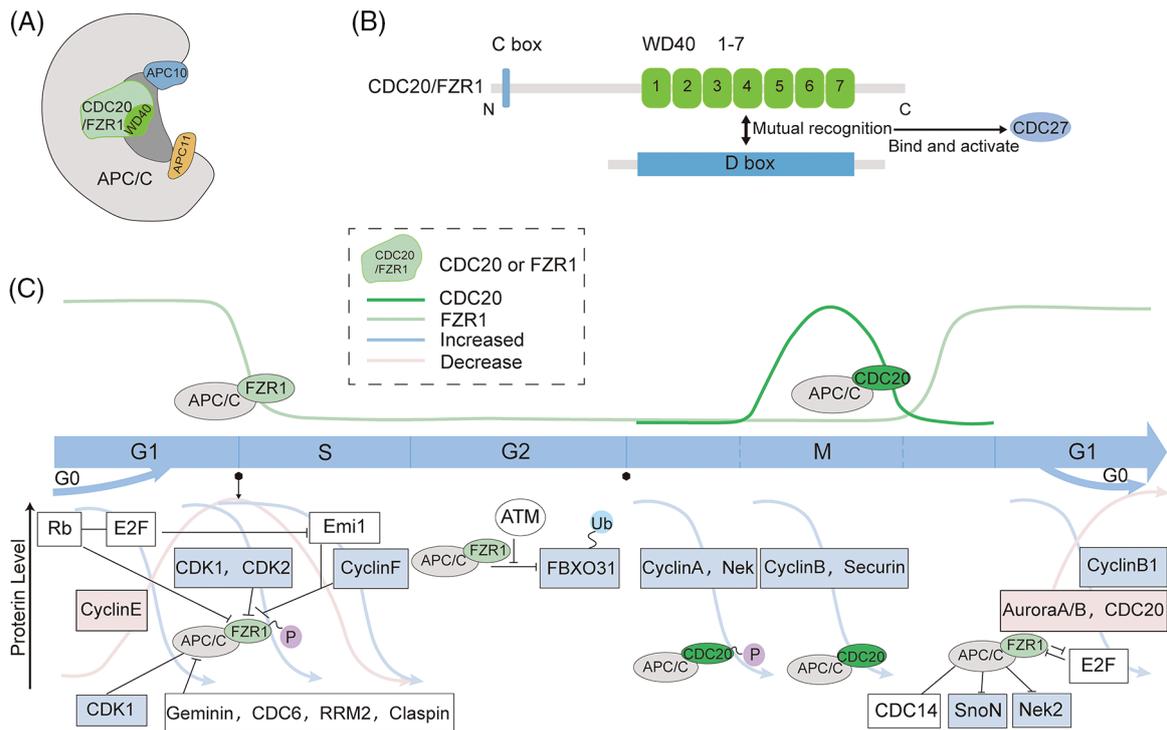
FIGURE 1. Schematic structure and intra-cycle regulation of fizzy-related protein homolog 1 (FZR1). (A to B) FZR1 mainly comprises a β-propeller with a seven-leaf WD40 repeat and a C-box containing significant functional motifs. (C) APC/C^{FZR1} activity regulates the cell cycle process and fluctuates throughout the cell cycle. APC/C^{FZR1} maintains its stability as a tumor suppressor after cells enter the G1 phase. In the G1 phase, Rb represses E2F by binding to transcription factors and regulates APC/CFZR1 through a double negative feedback mechanism of Emi1. Cyclin E accumulates and activates Cdk2, followed by CDK1 and Cdk2 to phosphorylate FZR1, and geminin, CDC6, RRM2, and claspin to inactivate APC/C^{FZR1}, allowing the cell to enter the S phase. Upon inactivation of APC/C^{FZR1} by Cdk2, Emi1 is converted from a substrate of APC/C to its inhibitor. Although FZR1 levels are low during G2, it can still act through the ubiquitinated tumor suppressor FBXO31. However, this interaction is disrupted if FBXO31 is phosphorylated by the DNA damage response kinase ATM. Cyclin B degradation leads to CDK inhibition, dephosphorylation, and FZR1 activation. APC/C^{FZR1} initiates the degradation of various proteins, such as CDC20 and Aurora A/B, during prophase and telophase. At the end of mitosis, APC/C^{FZR1} inactivates APC/C^{CDC20} and regulates late spindle dynamics and cytoplasmic division. Thus, APC/C^{FZR1} is activated in late mitosis and controls proliferation and differentiation decisions during the G1 phase. The Wnt and TGF-β pathways are conserved extracellular signaling pathways that regulate different developmental and physiological processes in multicellular organisms. FZR1 regulates the pathway by regulating cellular SnoN transcription factors and Nek2 degradation. APC/C^{CDC20} content increases during mitosis and regulates the cell cycle through cytokinesis. This partially inhibits the substrate recruitment of APC/C activated by CDC20, but APC/C^{CDC20} can further degrade various pro-term and mid-term proteins such as cell cycle A, cell cycle B, and securin, thereby mediating chromosome segregation and initiating mitosis out.

and APC/C^{FZR1}, which regulate the targeting of APC/C to specific substrates. The disruption box is a pattern frequently found in the APC substrate, but others may also be recognized by APC/C^{CDC20} or APC/C^{FZR1} (Harper *et al.*, 2002) (Fig. 1A).

In this review, we present the role of FZR1 in tumors and discuss the feasibility of targeting FZR1 in cancer therapy. First, we introduce the function of FZR1 as an APC/C coactivator in regulating the tumor cell cycle, differentiation, genome stability, and DNA damage of tumor cells in recent years. The underlying mechanism and potential of FZR1 as target genes in tumor therapy are also discussed.

Functions of Fizzy-Related Protein Homolog 1

There is a high homology between Drosophila fizzy (fzy) and eukaryotic fizzy-related (fzr) (Sigrist and Lehner, 1997). The most classical function of FZR1 is to regulate the cell cycle in association with APC/C, and its general function is similar to that of CDC20, but it is mainly active in late

mitosis, and G1 phase (Fig. 1C). CDC20 and FZR1 are substrate-specific adapters of APC/C during the cell cycle and can function as targets of the spindle assembly checkpoint (SAC) (Pines, 2011; Primorac and Musacchio, 2013). The substrate of CDC20 is mainly a mitogenic protein, which functions at the initiation of mitosis by APC/C^{CDC20}, and phosphorylation of APC/C is critical for its mitogenic regulation (Kraft *et al.*, 2003).

In cell progression, APC/C^{CDC20} is present at the onset of mitosis until the onset of anaphase (Fig. 1C). Early in mitosis, CDC20 becomes more active and binds to phosphorylated APC/C, leading to the degradation of prometaphase substrates cyclin A and Nek2 (Kramer *et al.*, 2000; Kraft *et al.*, 2003; Qiao *et al.*, 2016; Zhang *et al.*, 2016). Once all chromosomes have reached proper microtubule double orientation at metaphase, APC/C^{CDC20} degrades securin and cyclin B, facilitating the cell transition to anaphase (Fig. 1C). Degradation of cyclin B eliminates cyclin-CDK activity at anaphase and enables APC/C^{FZR1} activation, which disrupts other mitotic regulators, including CDC20 (Listovsky *et al.*,

2004). APC/C^{FZR1} appears in late G1 and promotes efficient exit from mitosis and maintenance of the G1 state (Fig. 1C). In addition, FZR1 plays a crucial role in controlling proliferation, differentiation, and maintenance of genome integrity.

In early mitosis, CDC20 begins to become more active and binds to phosphorylated APC/C, dislocated from the C-box binding site of the inhibitory region Apc1-loop300, allowing CDC20 to bind, leading to degradation of the prometaphase substrates cyclin A and Nek2 (Kraft et al., 2003; Qiao et al., 2016; Zhang et al., 2016; Yamano, 2019). The autoinhibitory loop in APC1 prevents CDC20 from binding to APC/C, and phosphorylation alleviates this inhibition (Qiao et al., 2016). Interestingly, CDC20 binds to phosphorylated APC/C, whereas its phosphorylation status is dispensable for its binding to APC/C. However, due to competition from FZR1, APC/C^{CDC20} is maintained at a low level for most of the cell cycle (Fig. 1C); as a result, APC/C lacks sufficient phosphorylation to form an activated APC/C^{CDC20} complex, which makes FZR1 more competitive to bind APC/C (Fujimitsu et al., 2016). At the onset of anaphase, APC/C requires the activating protein CDC20 to target safety proteins and cyclin B1 for proteasome-dependent degradation but then relies on the CDC20-associated protein FZR1 to remain active until the onset of the next S phase (Sigl et al., 2009). Degradation of cyclin B eliminates cyclin-CDK activity at anaphase and allows APC/C^{FZR1} activation, disrupting the remaining mitotic regulators, including CDC20 (Brandeis and Hunt, 1996; García-Higuera et al., 2008). APC/C^{FZR1} appears in the G1 phase of late cells and promotes efficient exit from mitosis and maintenance of the G1 state.

Late in mitosis, dephosphorylation of FZR1 activates APC/C, allowing it to continue ubiquitinating mitotic cycle proteins and new targets in place of CDC20 to drive cell mitosis into G0/G1 (Peters, 2006; Skaar and Pagano, 2008; Pines, 2011). APC/C^{FZR1} activity is present throughout G1 until FZR1 is inactivated at the G1 to S transition through degradation, phosphorylation, and binding of Emi1. At the G1/S transition, FZR1 is phosphorylated, leading to its dissociation from APC/C. After completion of mitosis, APC/C remains active until the next S phase (Brandeis and Hunt, 1996; Skaar and Pagano, 2008); the inactivation of APC/CFZR1 is required for the G1 to S transition of cells (Li and Zhang, 2009). DNA replication in the S phase, requires cyclin-dependent kinase 2 (CDK2) activation. The changes in regulatory trigger mechanism, APC/C^{FZR1} inactivation, and Emi1 content are the key points to control permanent cell cycle entry. Emi1 performs rapid and irreversible inactivation of APC/C^{FZR1}. After its inactivation, it cannot revert to quiescence (Cappell et al., 2016, 2018).

In mammals, the placenta is an essential organ of the embryo, and deletion of FZR1 can affect placental development. In the placenta, polyploid giant cells are acquired by endoreplication, and placental giant cells fail to form in the absence of FZR1, suggesting that APC/C^{FZR1} is required for endoreplication of DNA and that to undergo endoreplication, cells must not enter mitosis after one round of DNA synthesis (García-Higuera et al., 2008; Li et al., 2008).

Fizzy-Related Protein Homolog 1 Is a Master Regulator of the G1 Phase and the Quiescent G0 Phase in Tumor

Cell cycle control occupies a central place in the complex rules that govern the various processes within our cells, and the exit of mitosis requires the degradation of mitotic cyclins (Amon et al., 1994). In the past, many studies have shown the role of FZR1 in the cell cycle. Despite substrate overlap, APC/C^{CDC20} mainly regulates metaphase and postmitotic transitions and regulates mitotic exit, while APC/C^{FZR1} is active at the end of mitosis and G1 phase (Fig. 1C). FZR1 can regulate the rapid degradation of mitotic cyclin in the cell cycle (Sigrist and Lehner, 1997). Phosphorylation and dephosphorylation of FZR1 regulate the binding, activation, and separation of APC/C with CDC20 and FZR1 (Kramer et al., 2000) Phosphorylated FZR1 is found to be limited in binding to APC/C during the cell cycle, thereby blocking the activation of APC/C (Kramer et al., 2000). In FZR1 deficient cells, the G1 phase is shortened, and the S phase is prolonged. The retinoblastoma protein (pRb) is another G1/G0 regulatory factor. FZR1 has been confirmed to bind to pRb with synergistic effects in the G1/G0 phase (Kernan et al., 2018). The inactivation of FZR1 is critical for cells to enter the S phase (Cappell et al., 2018). During the G1 phase, retinoblastoma (Rb) inhibits E2F by binding to transcription factors (Yao et al., 2008). Accumulation of cyclin E activates Cdk2, which phosphorylates FZR1, inactivates APC/C^{FZR1}, and causes cells to enter the S phase (Yao et al., 2008). After Cdk2 inactivates APC/C^{FZR1}, Emi1 changes from a substrate of APC/C to its inhibitor, which inactivates Emi1 irrepressibly, making it unable to return to the G0 phase (Cappell et al., 2016, 2018), allowing cells to enter the S phase, CDK inhibitors cooperate with FZR1 to maintain the G0/G1 phase state (Kim and Bonni, 2007). The inactivation of CDK1 phosphorylates FZR1 and binds APC/C to degrade CDC20. APC/C^{FZR1} is a key factor in G1/G0 regulation by keeping cells in the G1 phase or entering the G0 phase (Kernan et al., 2018). Moreover, several replication regulators of APC/C^{FZR1} are disrupted during the G1/S transition, including Geminin (McGarry and Kirschner, 1998), CDC6 (Petersen et al., 2000), RRM2 (Chabes et al., 2003), and claspin (Bassermann et al., 2008), which can inactivate APC/C^{FZR1}. Although the activity of APC/C^{FZR1} decreases after the G1/S transition, there is a role for FZR1 in the G2 phase, and FBXO31, an adapter for the E3 ligase SCF, has been suggested to function as a substrate for APC/C^{FZR1}, with the two being dependent on both AKT and ATM kinases, among them, the oncogene AKT ubiquitinates FBXO31 via APC/C^{FZR1} (Choppara et al., 2018). AKT can regulate APC/C^{FZR1} recognition of SKP2 and cyclin F (Wei et al., 2004; Choudhury et al., 2016). In the G2 phase, FZR1 and CDC20 accumulate, but APC/C^{FZR1} remains inactive in late mitosis due to the inhibition of the protein Emi1 and phosphorylation of cell cycle protein-dependent kinases (CDKs), which inhibit

FZR1 binding to APC/C in late prophase. Unlike Emi1 regulation, the accumulation of APC/C substrate proteins can be promoted by rapid dissociation of FZR1 after human cytomegalovirus (HCMV) infection of quiescent cells (Wiebusch et al., 2005). In FZR1-deficient cells, cyclin B1 is efficiently degraded during mitosis, with a shortened G1 phase and prolonged S phase, causing a proliferation defect, and is largely independent of p53 (Sigl et al., 2009) (Fig. 1C). APC/C^{FZR1} can conduct intracellular and extracellular cascade signals. For example, the SnoN transcription factor is thought to be a substrate of FZR1; FZR1 may directly interact with SnoN and Smad3 to induce SnoN degradation, which makes APC/C-dependent, and thus promotes TGF-β-induced transcription (Stroschein et al., 2001; Wan et al., 2001) (Fig. 1C).

The property of FZR1 is also seen in diseases. In breast cancer, FZR1 mediates cisplatin-induced apoptosis by weakening phosphorylation at the ser15 position involved in p53 stability, which can lead to breast cancer cell cycle arrest (Liu et al., 2020). While shortening the G1 phase, FZR1 knockout also leads to the accumulation of DNA damage in progenitor cells, and premature entry of cells into the S phase can also lead to p53-mediated apoptosis and a decrease in the number of progenitor cells (Delgado-Esteban et al., 2013; Eguren et al., 2013). FZR1 can be used as a substrate for CDK4/6, phosphorylating the Ser714 and Thr719 sites located between the A and B regions of the pocket to reverse the cytokinesis blocking effect induced by CDK4/6-specific inhibitors (The et al., 2015). In addition, FZR1 directly interacts with Src in breast cancer cells, enhances binding between the N-terminal and c-terminal structural domains of Src, locks Src in a closed conformation to inhibit Src, and can induce phosphorylation at the Y419 position with enhanced binding capacity in the G2 phase (Han et al., 2019). CDC27 is a core component of the APC/C that anchors FZR1 to the ubiquitinated substrate on APC/C (Peters, 2006). Concurrently, its dysregulation can lead to premature activation of the APC/C^{CDC20} complex and subsequent abnormal degradation of FZR1, resulting in abnormal mitosis and aneuploidy (Link et al., 2016). FZR1 binds directly to the LxCxE-binding vesicle of pRB via a cysteine residue in its LxCxD motif, but this motif does not affect the association of FZR1 with core APC/C. When this interaction is significantly disrupted, polo-like kinase 1 (PLK1) and S phase kinase-associated protein 2 (SKP2) accumulate, and the expression of the proteins p27Kip1 and p21Cip1 are down-regulated. Defects exist in the G1 cell cycle (Ramanujan et al., 2021). In neurological disorders, Cdk5 (cyclin-dependent kinase 5) can be transported to the cytoplasm in response to neuronal stress to eliminate its cell cycle inhibitory activity. Neuronal re-entry into the S phase is ubiquitinated by the E3 ligase APC/C^{FZR1}. Ubiquitinated Cdk5 is then rapidly degraded by the proteasome, and subsequently, it is ubiquitinated by FZR1 and rapidly degraded, which plays a key role in the protective function of many neurodegenerative diseases (Zhang et al., 2012) (Fig. 1C). Moreover, APC/C^{FZR1} controls casein kinase 1δ (CK1δ) levels to regulate the proliferation and cell cycle exit of developing mouse cerebellar granule cell progenitors

(GCPs) (Penas et al., 2015). In the hematopoietic system, Recent studies have revealed new roles of FZR1 in the blood system. FZR1 haploinsufficiency in Fzr1 mice impairs hematopoietic stem cell (HSCs) quiescence and self-renewal, and FZR1 deficiency impairs RUNX1 ubiquitination in patients with severe aplastic anemia. This leads to RUNX1 protein accumulation and disturbs the quiescence of hematopoietic stem cells in patients with severe aplastic anemia (Zhou et al., 2022). The progression of replication forks in FZR1-deficient fetal hepatocytes is slowed, resulting in a delayed G2/M cell cycle (Cuadrado et al., 2022). In the G1 phase of the cancer stem-like cells (CSC), hyperphosphorylation of FZR1 itself can decrease APC/C^{FZR1} activity, mainly because APC/CFZR1 destroys borealin protein during the G1 process. Throughout the cell cycle, the levels of boreal in Aurora B remained constant (De et al., 2019). Functional inhibition of tank binding kinase 1 (TBK1) increases the association of FZR1 with APC1, leading to increased mitotic aberrations (Maan et al., 2021). Progression of the replication fork is slowed in FZR1-deficient fetal hepatocytes, resulting in a G2/M cell cycle delay (Cuadrado et al., 2022). FoxM1 haploinsufficiency can improve muscle regeneration in Fzr1 knockout mice by addressing the depletion of quiescent muscle satellite cells (SCs) pool through the promotion of SCS to enter the cell cycle (Chen et al., 2020). These results confirm the role of FZR1 in the cell cycle, including its interaction with CDC20 protein interaction and other cycles, and change the content to influence the cell cycle process, and potentially lead to either delayed or accelerated cell cycle process depending on FZR1 (Fig. 1C).

Fizzy-Related Protein Homolog 1 Regulates Differentiation via the Cell Cycle

The process of cell differentiation occurs when the stem cells of multicellular animals divide and give rise to various cell types. During this process, the gene expression of the daughter cells is controlled. A critical component that regulates G1 residence on a bistable switch is also used to hold the differentiation component, causing G1 to be coordinately arrested and differentiated. As was previously mentioned, FZR1 inactivation during the G1 phase is crucial for starting a new cell cycle. Several mechanisms regulate cell cycle departure and differentiation. Some studies suggest that FZR1 not only participates in the cell cycle but may also encourages cell cycle exit and differentiation of specific cell types.

The role of FZR1 in differentiation has been described earlier. During yeast cell division, Cdk inhibitors are downregulated. FZR1 is activated by the pheromone signaling pathway and binds to and inhibits Cln-Cdc28 kinase, resulting in G1 cell cycle arrest (Peter and Herskowitz, 1994). When primary neural stem cells are differentiated into neurons, the presence of all-trans retinoic acid-induce an up-regulation of FZR1 expression and down-regulation of downstream substrate Id2 expression, indicating the control role of FZR1 in the differentiation of neural stem cells into neurons (Yao et al., 2010). Retinoic
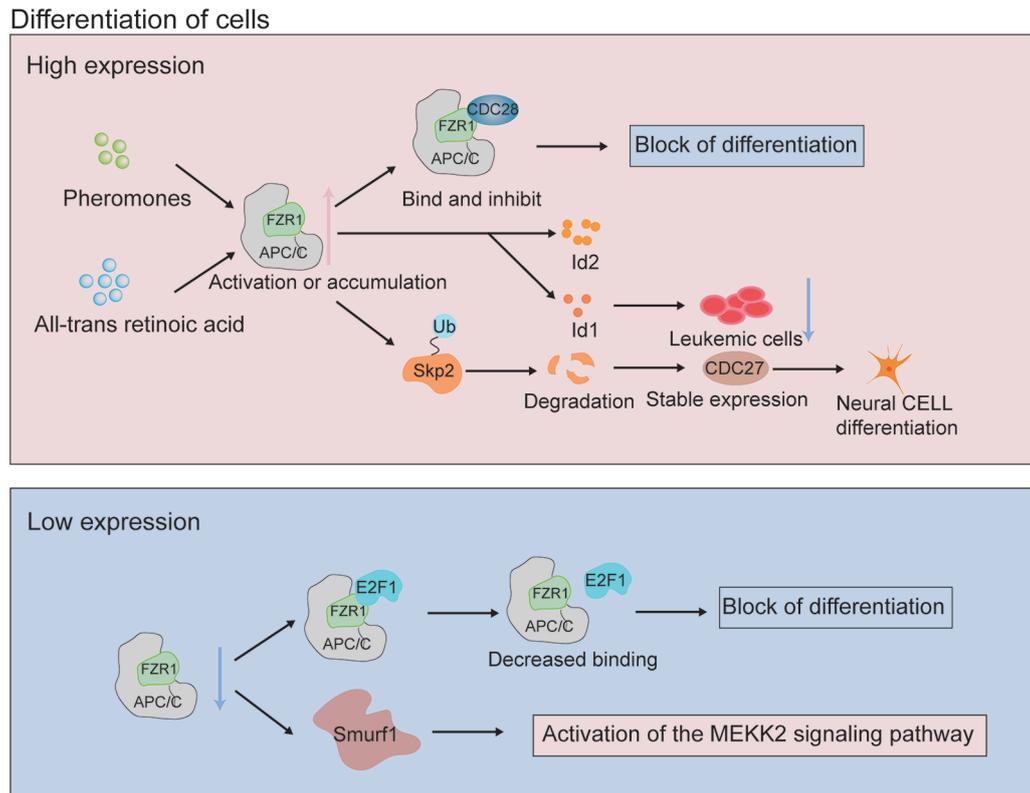
FIGURE 2. Mechanism of action of fizzy-related protein homolog 1 (FZR1) in tumor differentiation. Enhanced FZR1 expression is induced by CDK inhibitor and all-trans retinoic acid stimulation. Cdk inhibitors negatively modulate the role of FZR1 in differentiation, activated by the pheromone signal transduction pathway to bind and inhibit Cln-Cdc28 kinase. The role of FZR1 in differentiation upregulates the cyclin-dependent kinase p27. The interaction of FZR1 controls p27 stability by targeting Skp2 ubiquitination degradation, which promotes cell cycle exit and regulates differentiation. Inhibition of FZR1 prevents its binding to E2F1, which together regulate post-translational modification and down-regulate E2F1, producing a response to differentiation signals.

acid can stimulate neuroblastoma cells by controlling FZR1 nuclear accumulation, Skp2 degradation and p27 accumulation to cause cell cycle arrest and differentiation, and encourage the differentiation of human SH-SY5Y neuroblastoma cells (Cuende et al., 2008) (Fig. 2).

FZR1 may also be as crucial in differentiation as cyclin-dependent kinase p27 or the tumor suppressor retinoblastoma protein (pRb), primarily because pRb interacts with APC/C^{FZR1} to control the stability of p27 by targeting Skp2 ubiquitination degradation and promoting pRb-mediated cell cycle exit (Binné et al., 2007). The Id gene is expressed in human acute myeloid leukemia cells and inhibition of Id1 expression can prevent the growth of leukemia cell lines; therefore, FZR1 may be involved in hematopoietic stem cell differentiation by controlling Id protein and other likely targets. This is because Id is necessary for the proliferation of leukemia cells. Myeloid cancers may occur if the expression is dysregulated (Ji et al., 2008). Like FZR1, ras also interacts with pRb to influence differentiation, contribute to tumorigenicity, and may even lead to metastatic disease and increase the likelihood of overlapping transformation pathways (Fig. 2). FZR1 is a G0/G1 regulator, which can control the differentiation of hematopoietic stem cells into mature progenitor cells of the lineage and regulate the self-renewal of hematopoietic stem cells (Ishizawa et al., 2011). Abnormal expression of E2F1 can promote non-melanoma skin cancer, and FZR1 binds to

E2F1 in keratinocytes. Inhibition of FZR1 prevents its binding to E2F1, co-regulating post-translational modification, and down-regulating E2F1, resulting in a response to differentiation signals (Singh and Dagnino, 2017).

In the Fzr1 knockout mouse model, genetic ablation of Fzr1 impaired the ability of APC/C to promote neurogenesis by delaying progenitor cell exit from the cell cycle (Delgado-Esteban et al., 2013). Loss of FZR1 can induce SmURF1-induced activation of the MEKK2 signaling pathway and induce osteoblast differentiation (Wan et al., 2011). While the significance of many of the described mechanisms has yet to be confirmed through in vivo models, FZR1 plays a crucial role in coordinating proliferation and differentiation.

Fizzy-Related Protein Homolog 1 Regulates the G2/M Checkpoint upon DNA Damage

High-fidelity DNA replication and chromosome separation are necessary to preserve genome integrity and prevent carcinogenesis. The regulatory role of FZR1 on the G2/M phase transition has been confirmed earlier. In G2 phase, CDK1 phosphorylates CDC20 and activates CDC20 (Kramer et al., 2000; Kraft et al., 2003), and APC/C^{CDC20} degrades Nek and cyclinA in prometaphase (Boekhout and Wolthuis, 2015), securin and cyclinB1 in metaphase (Fig. 1C). FZR1 remains silent during G2 phase and early
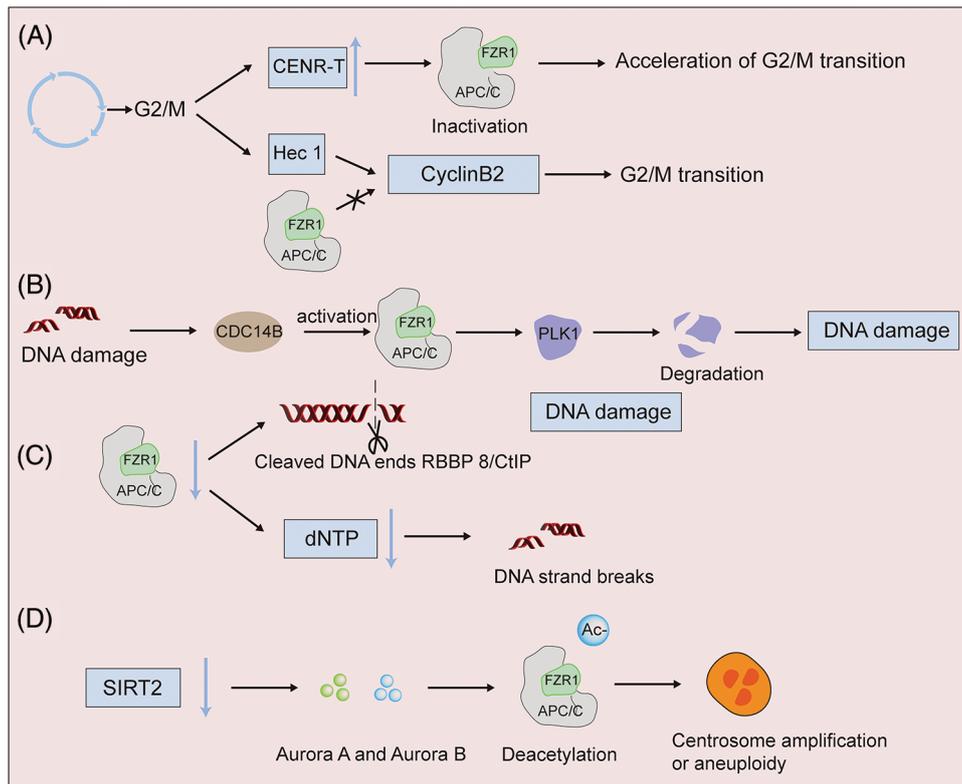
FIGURE 3. APC/C^FZR1^ activity has different expressions in DNA damage. (A) FZR1 regulates the G2/M phase transition. Overexpression of CENR-T inactivates FZR1 and accelerates the G2/M phase transition of oocytes. Hec1 antagonizes the stability of cyclin B2 with APC/C^FZR1^, preventing it from converting to the G2/M phase (B to D). High-fidelity DNA replication and chromosome segregation are required to maintain genome integrity and prevent carcinogenesis. Knockdown of FZR1 results in binucleated or multinucleated cell aberrations, and acute or permanent deletion of FZR1 increases DNA fragmentation and protein cleavage. After DNA damage, PLK1 ubiquitination is induced, which in turn affects cell differentiation.

mitosis due to phosphorylation (Kramer *et al.*, 2000; Peters, 2002), whereas in anaphase, CDC14 dephosphorylates FZR1 (Visintin *et al.*, 1998) (Fig. 1C) and ubiquitinates CDC20 (Huang *et al.*, 2001), Aurora kinase (Littlepage and Ruderman, 2002), polo-like kinase 1 (PLK1) ensure mitotic exit.

In oocytes, CENR-T overexpression inactivates FZR1 and accelerates the G2/M1 phase transformation of oocytes (Wang *et al.*, 2020). CenpH competed with APC/C^FZR1^ in the centromeric inner plate, affecting cyclin B1 stability, resulting in the downregulation of MPF activation and regulating G2/M transition (Zhang *et al.*, 2017). In addition, Hec1 can stabilize cyclin B2, while APC/C^FZR1^ can destroy cyclin B2 and prevent cyclin from transforming into the G2/M phase (Gui and Homer, 2013). FZR1 was found to be an essential component of the G2 checkpoint induced by DNA damage in chicken B-cell lymphoma cell lines. After DNA damage, FZR1-APC complex was activated to induce G2/M transition regulator degradation and G2 arrest (Sudo *et al.*, 2001) (Fig. 3A). At the same time, after DNA damage in the G2 phase, Cdc14B activates APC/C^FZR1^ and promotes the degradation of mitotic kinase Plk1, thereby regulating the G2 DNA damage response checkpoint (Bassermann *et al.*, 2008). The deficiency of FZR1 can cause the accumulation of DNA damage in mature red blood cells (Cuadrado *et al.*, 2022). The loss of Fzr1 *in vivo* increases the DNA damage in B-ALL cells, and long-term loss can lead to drug resistance (Ishizawa *et al.*, 2017). After DNA damage, FZR1 is required at the G2 DNA damage checkpoint; its dephosphorylation and rebinding to APC/C lead to ubiquitination of PLK1, preventing entry into mitosis. As an adapter for APC/C (Fig. 3B), FZR1 targets the DNA terminal excision factor RBBP 8/CtIP for ubiquitination and subsequent proteasome degradation. The regulation of the turnover of RBBP 8/CtIP proteins can possibly play a role in DNA damage response by facilitating DNA double-stranded repair via error-prone non-homologous terminal junction compared to error-free RBBP 8-mediated homologous recombination (Fig. 3C). The discovery that FZR1 deficiency decreased dNTP levels and increased DNA fragmentation strongly suggested that abnormal replication was the cause of genomic instability in primary mouse embryo fibroblasts, as was the finding that acute depletion or permanent ablation of FZR1 limited the progression of replication fork mobility (Fig. 3C). These findings underscore the importance of FZR1 activity in reducing replication stress and the subsequent chromosomal breakage leading to tumor development (Garzón *et al.*, 2017). Some studies have shown that decreased levels of silence information regulator 2 can lead to increased levels of the mitotic regulators Aurora-A and Aurora-B, which deacetylates APC/C^FZR1^ to regulate APC/C activity. Results in centrosome amplification, aneuploidy, and mitotic cell death, suggest that silence information regulator 2 acts to suppress tumors by regulating mitosis and genome integrity (Kim *et al.*, 2011). In future work, it will be interesting to

understand how oncogene-induced genomic instability is promoted when DNA damage caused by FZR1 deletion occurs (Fig. 3D).

Summary and Outlook

FZR1 participates in a variety of processes, and the ability of FZR1 to regulate the cell cycle is well-known and has a clear connection with cancer. Since FZR1 regulates many events in the cell cycle, it is advantageous for cancer cells to maintain a certain level of activity. While some oncogenic targets of FZR1 can enhance activity through amplification or other FZR1-independent mechanisms, which may contribute to tumorigenesis, overall, combined deregulation of multiple pathways by partially reducing FZR1 function appear sufficient to create a permissive environment for tumor cell evolution. However, its function in regulating cancer is not limited, and here we discuss more recent publications and highlight current studies that have improved our understanding of FZR1 and cancer. Specifically, because FZR1 plays a role in tumorigenesis and progression, such as regulation of tumor quiescence, regulation of differentiation through the cycle and the G2/M checkpoint through ubiquitination of PLK1 upon DNA damage. Recently, the robust mechanism of APC/C^{FZR1} relevant to carcinogenesis was shown. As described earlier in this paper, low expression or low activity of FZR1 is associated with cell cycle dysregulation, cell differentiation, genomic instability, DNA damage, and the regulation of many cancer-related pathways. However, although FZR1 is generally considered to be a tumor suppressor, in several cancers, FZR1 may be overexpressed at the RNA and protein levels. Like reduced activity, elevated FZR1 activity is associated with DNA damage and regeneration, which may contribute to tumorigenesis. However, given that multiple mechanisms destabilize the FZR1 protein, elevated RNA may not imply high protein levels. Overall, the singular deficiency in FZR1 activity appears to be achieved in cancer through a potential combination of multiple mechanisms of activity, mainly post-translational, which complicates the development of strategies to use FZR1 activity as a cancer treatment. FZR1 expression is not necessarily an indication of activity in the context of the cell cycle. The expression of FZR1 peaks in G2 and M but remains inactive during anaphase of mitosis. Since the expression of many APC/C substrates also peaks during the G2 and M, the positive correlation between FZR1 protein and APC/C substrates observed in the TMA analysis may reflect the cell cycle status of tumor cell proliferation. Interestingly, in addition to the function of FZR1 in tumors, recent studies have also found that there are also some mutations or deletions that can lead to the gain or loss of FZR1 function during cancer growth. In the GS-Wnt model of gastric carcinoid, mutations in FZR1 lead to a structural shift to a grape-like form (Seidlitz et al., 2019). Mutated genes that initiate FZR1 in the Wnt subtype of medulloblastoma tumorigenesis (Robinson et al., 2012). However, increasing evidence suggests that enhancing APC/C^{FZR1} activity may contribute to the activity of current therapeutic strategies, including inhibition of Akt, Src, and

potentially CDKs. CDC20 remains an important clinical target of APC/C because it is a coactivator required for mitotic exit.

The study of FZR1 for nearly 25 years since its discovery has revealed a conserved mechanism of APC/C^{FZR1}-dependent cell cycle regulation from yeast to humans. It is now clear that FZR1 plays a key role in cell division, stem cell regulation, neuronal processes, DNA damage, and tumorigenesis. Further validation of the newly developed APC/C^{FZR1} small molecule inhibitors using various animal disease models based on basic research is expected to initiate a new era of APC/C^{FZR1}, and a better understanding of these tumorigenic mechanisms could both lead to a better understanding of cancer and the development of more sophisticated targeted cancer therapies.

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