CDK9: a key player in Cancer and Other Diseases

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ABSTRACT

Cyclin-Dependent Kinase 9 (CDK9) is part of a functional diverse group of enzymes responsible for cell cycle control and progression. It associates mainly with Cyclin T1 and forms the Positive Transcription Elongation Factor b (p-TEFb) complex responsible for regulation of transcription elongation and mRNA maturation. Recent studies have highlighted the importance of CDK9 in many relevant pathologic processes, like cancer, cardiovascular diseases and viral replication. Herein we provide an overview of the different pathways in which CDK9 is directly and indirectly involved.

KEYWORDS

CDK9; cancer pathway; cyclin; drug targeting; transcription regulation; kinase; viral replication.

1. INTRODUCTION

Cyclin-Dependent Kinases (CDKs) are a family of enzymes that work in association with another family of regulatory subunits called Cyclins. Together they work to form functional heterodimeric complexes responsible for the control of several cellular vital functions including proliferation, differentiation, DNA repair and apoptosis. CDKs are proline-directed serine/threonine protein kinase; their well-characterized structural features and molecular mechanisms have been the inspiration for the design of new inhibitors becoming one of the most attractive pharmacological targets in the development of new antiproliferative drugs nowadays. CDKs were initially discovered as regulators of cell cycle transitions, ensuring timely and accurate cell replication by arranging DNA replication and cell division. As the functional diversity of CDK members has been previously understood, it can be said that not all CDKs played a direct role in synchronizing the cell cycle progression. However, there are also CDKs involved in various mechanisms of RNAP II transcription regulation, where CDK9 is the main actor in this process [Romano and Giordano, 2008]. Here we provide an overview on CDK9 biology, physiopathology and its potential for drug targeting.

2. BIOLOGY OF CDK9

CDK9 maps to chromosome 9q34.1 and, like the other transcriptional CDKs, it has a conserved two-lobed structure in its active site . It was initially isolated as a 42 kDa cdc2 - like serine/threonine kinase ($CDK9_{42}$), and it was referred to PITALRE because of its homology with the PSTAIRE motif of cdc2 [Grana et al., 1994]. However, in 2003, an additional product that encodes a 55 kDa protein ($CDK9_{55}$) [Morales and Giordano, 2016] was identified, which has an additional 117 amino acid residues at the amino terminus. This makes it 13kDa heavier than the

original one. $CDK9_{55}$ is encoded with the same gene as $CDK9_{42}$, but it is regulated by a different promoter region. Also, it has been less reported in literature [Morales and Giordano, 2016]. Both are transcriptional CDKs and they act in the regulation of gene expression by allowing transcription and maturation of messenger RNA (mRNA) [Morales and Giordano, 2016].

Generally, CDK9 associates with a cyclin, to form the catalytic subunit of the Positive Transcription Elongation Factor b (P-TEFb), which is a two-unit molecule needed for Polymerase II proper function. This association promotes the rotation of the molecule and induces the activation by autophosphorilation of the conserved T Loop [Romano and Giordano, 2008]. The different phosphorylations that CDK9 had encountered may have influenced its enzyme activity, as well as P-TEFb interactions with other regulatory factors. Both CDK9 isoforms form complexes with cyclins, including any of the T1, T2a, T2b, and K cyclins, producing eight distinct heterodimers. However, the functional significance of the expression of distinct isoforms and the specificity of their association to different cyclins has not been well clarified yet.

2.1 CDK9 AND ITS RELATION TO THE C-TERMINAL DOMAIN (CTD)

Transcription is one of the fundamental biological process by which cells build their specific proteins, and RNA polymerase type II (RNA pol II) is the main effector of this process (Figure 1). With the help of several transcriptional factors (TF) and various other proteins (like CDKs), RNA pol II goes through transcription in 5 sequential steps: i) Preinitiation, ii) Initiation, iii) Promoter Clearance, iv) Elongation, and v) Termination. To begin, DNA is unwound in the area where the desired gene is going to be transcribed. RNA pol II and transcriptional factors are called-in to form the Pre-Initiation Complex (PIC). RNA pol II is conformationally changed by the CDKs actions and the promoter region get cleared up, promoting the elongation phase of mRNA. The process

continues until it reaches its termination point [Krasnov et al., 2016]. Each step is executed by a complex array of protein interactions far from the scope of this review.

In eukaryotes and most prokaryotic organisms, after a gene transcription has been activated (**Step 1**, Figure 1), Transcription Factor IID (TFIID) binds first to the promoter sequence of the DNA template **(Step 2)** and recruits GTFs and the Mediator Complex (MC)**(Step3)**, which is the largest co-activator of mRNA synthesis. MC recruits RNA pol II **(Step 4),** and together they form the PIC (see Figure 1) [Tsai et al., 2013], a complex in an "inactive" or "closed" state. PIC is activated by the Transcription Factor IIH (TFIIH), which stabilizes RNA pol II **(Step 5)**, then opens the DNA strand at the Transcription Start Site (TSS) and approaches the DNA template to the RNA pol II active site, forming the PIC "open" form [He et al., 2013]. Once PIC is activated, RNA pol II starts recruiting the first nucleoside triphosphates (NTPs) according to the DNA template sequence, and proceeds with the formation of the first phosphodiester bound **(Step 6)**. At the same time, the TFIIH forms with the CDK7/cyclin H, and together with the catalytic subunit CDK8 of the MC, phosphorylate the Ser5/Ser7 and Ser2/Ser5 residues of the heptad tandem repeat region of the CTD **(Step 7)** [Schuller et al., 2016]. The phosphorylation of the Serine residues allows the initiation of the elongation process that is denominated *promoter clearance* **(Step 8)**. It occurs with a concomitant partial disassemblage of the PIC, which can be reused in future transcription processes [Yudkovsky et al., 2000].

Around 20-100 bp down-stream from the TSS, the NTEFs, DRB Sensitivity Inducing Factor (DSIF) and Negative Elongation Factors (NELF) **(Step 9)** all work together, participating in the normal pausing of RNA pol II activity. The normal pausing of RNA poll II activity is a short phenomenon that occurs to regulate the rate of transcriptions and allows the recruitment of enzymes involved in the capping and splicing machineries of mRNA [Gilchrist et al., 2010]. Transcription-positive elongation factors, like P-TEFb, are needed to reverse the effect of negative

elongation factors that associate with Pol II during initiation. Bromodomain-containing protein 4 (BRD4) recruits the P-TEFb complex (previously activated by the MC, and not shown on Figure 1) and allows the transcription process to continue by phosphorylating the CTD of RNA pol II through its catalytic subunit, the CDK9. *In vivo* and *in vitro* studies have shown a primary phosphorylating effect on the Ser5 residue of every CTD heptapeptide repeat of the RNA pol II that has being pre-phosphorylated at Ser7 (CDK7) [Czudnochowski et al., 2012]. Once this step is done, Ser2 is also phosphorylated by P-TEFb in a lesser amount and on the early elongation complex [Ghamari et al., 2013]. Concomitantly, CDK9 is also responsible of phosphorylating NELF and DSIF, and converting the later in a positive elongation factor and releasing the NELF from the complex **(Step 10)** [Czudnochowski et al., 2012; Schuller et al., 2016]. This step permits the transcription elongation, and is denominated Trascription Elongation Checkpoint Control (TECC). The process continues through elongation until transcription termination and final synthesis of mRNA **(Step 11)** [Laitem et al., 2015].

Figure 1. ***(1)*** *Transcription starts by activation of a gene.* ***(2)*** *First, the Transcription Factor II D (TFIID) attaches to the TATA-Box of the promoter region of the DNA template,* ***(3)*** *followed by General Transcription Factors (GTFs) and the Mediator Complex (MC), which is formed by several molecules like CDK8.* ***(4)*** *The RNA pol II is then recruited by the MC and the inactive Pre-Initiation Complex (PIC) is formed.* ***(5)*** *MC recruits also the Transcription Factor IIH (TFIIH), which joins the PIC to stabilize the RNA pol II and activate the PIC, which changes its conformation from closed to open to initiate the transcription process.* ***(6)*** *When the transcription starts, the first nucleotides of the new mRNA bound together.* ***(7)*** *CDK8 (part of the MC) and CDK7 (associated to TFIIH) phosphorylate the residues Serine 2 (Se2)/Se5 and Se5/Se7, respectively, to allow clearance of the promoter.* ***(8)*** *Shortly after, due to the phosphorylation of the Serine residues, the RNApolII is able to clear the promoter by releasing several GTFs and allowing transcription to continue.* ***(9)*** *The transcription process is regulated by DRB sensitivity-inducing factor (DSIF) and Negative Elongation Factor (NELF), which join the RNA pol II and induce a transcription pause.* ***(10)*** *To allow transcription elongation, CDK9 (part of pTEFb complex) comes in and phosphorylates DSIF, NELF and residues Se2 and Se5 of the CTD.* ***(11)*** *DSIF and NELF transform into Positive Elongation Factors (PEF) and stimulate the transcription elongation step.* ***(12)*** *Elongation continues until the termination of transcription and the full synthesis of mRNA.*

2.2 CDK9 RELATED INTRACELLULAR PATHWAYS

Besides the close relationship between CDK9, TFs, and RNA pol II involved in the transcription machinery, this CDK has many different intracellular responsibilities and diverse protein interactions. After its synthesis around 80% of CDK9 forms a heterodymer with Cyclin T1. The remaining 20% forms a complex with Cyclin T2A or Cyclin T2B, with presumable overlapping functions and expression patterns [De Luca et al., 2001]. Soon after CDK9 binds to the cyclin, an auto-phosphorylation process takes place and the Threonine residue 186 (Thr186) on the tip of the conserved T-loop in CDK9 acquires a phosphate group that consequents on the P-TEFb full catalytic activation [Li et al., 2005].

Following this Thr186 phosphorylation, the pTEFb complex is ready to control the elongation process. This activation must be tightly regulated. Between 50% and 90% of activated P-TEFb available in cells is sequestrated in a large intranuclear complex capable of inhibiting its kinase activity [Li et al., 2005]. This complex is formed by three parts: The first being *7SK snRNA*, a conserved small ribonucleoprotein synthetized by RNA pol III that binds directly with the Cyclin T1 of two P-TEFb [Haaland et al., 2003]. The second is Hexim 1 and Hexim 2 that interact directly with 7SK snRNA and Cyclin T1, and independently inhibits CDK9.[Romano and Giordano, 2008] This is then followed by the third part, which is LARP7; a Lupus antigen related protein 7 that binds to 7SK snRNA to provide stability to the final complex named 7SK snRNP [Matrone et al., 2015]. This final complex is capable to release the needed amounts of a ready-to-use P-TEFb [Li et al., 2005].

At this point, another important protein, BRD4, comes about. As figure 1 shows, this protein is activated by the MC during transcription and is the responsible of recruiting P-TEFb by binding to it after it dissociates from the 7SK snRNP. Following this, BRD4 binds to the acetylated lysine

residues in histone 3 (H3) and H4 of the active gene, and approaches active P-TEFb to the gene promoter, enabling its functions on the activated transcribing gene [Czudnochowski et al., 2012]. However, in few cases, P-TEFb does not interact with BRD4 for transcription activation. Instead, it joins several other potent Elongation Factors and forms the Super Elongation Complex (SEC). The SEC can be formed by different type of protein interactions between AFF1, AFF4, ELL1, ELL2, ENL and AF9, with P-TEFb working together to stimulate elongation of RNA Pol II during rapid transcription induction of immediate response genes [Luo et al., 2012]. The importance of P-TEFb function through SEC is easily understood under Heat Shock circumstances, where the rapid transcription of the Heat shock protein 70 (Hsp70) responds to an increased amount of SEC at the promoter region (Figure 2) [Luo et al., 2012].

At the same time, as P-TEFb stimulates transcription elongation, it promotes the recruitment of capping enzymes to the nascent pre-mRNA process that protects the pre-mRNA from degradation, and subsequently stimulates the action of the splicing enzymes [Guiguen et al., 2007]. There is a direct interaction between CDK9 and Pcm1, a caP-methyltransferase, important for the formation of the 7mG (7-methylguanosine) cap [Guiguen et al., 2007], and an indirect association with the CaP-Binding Complex (CBC), main actor in further recruitment of more 7mG enzymes [Gonatopoulos-Pournatzis and Cowling, 2014]. Once the 7mG cap is formed, the previously activated CBC favors the assembly of the splicing complex called Spliceosome [Gonatopoulos-Pournatzis and Cowling, 2014]. Furthermore, P-TEFb has been proposed to have an indirect role in the polyadenilation (poly-A) tail assembly and transcription termination [Laitem et al., 2015].

Figure 2. ***Inhibited/storage complexes****. They stimulate the transcription elongation by the positive activation of factor b (P-TEFb) kinase, or suppresses it within the 7SK small nuclear ribonucleoprotein (7SK snRNP). Heat shock protein 70 (Hsp70) stabilizes the CDK9 protein, responding to an increased amount of SEC at the promoter región;* ***Transcription complexes.*** *GATA4/p300 complex is required for DNA binding and transcription activation. CBC (Cap*

Binding Complex) is formed by RNA guanine-7-methyltransferase (Pcm1), which functions at the 5' transcription initiation site, and Pcm1, required for P-TEFb recruitment to chromatin and transcription elongation. BRD4 (Bromodomain-containing protein 4) is the responsible of recruiting p-TEFb by binding to it after it dissociates from the 7SK snRNP, enabling the activation of gene transcription. EAP (Elongation Assisting Proteins) is another transcriptional activator multiple complex. Super Elongation Complex (SEC) stimulates elongation of RNA Pol II during rapid transcription induction. ***DNA repair mechanism complexes:*** *p-TEFb directly activates p53, inactivates the retinoblastoma protein (pRb), and associates with Nuclear Protein Ataxia-Telangiectasia locus (NPAT), an important protein for histone gene expression.*

3. PHYSIOLOGY AND PHYSIOPATHOLOGY RELATED TO CDK9

CDK9 participates in transcriptional processes in most eukaryotic cells, including human tissues [Bagella et al., 1998]. The expression of CDK9 is ubiquitous, with considerable increases in different tissues and during different cell processes. Some of these include the development and growth of cardiac cell, hepatocytes, hematopoietic tissue, adipocytes, neurons and muscle cells, with major contribution to the progression and maintenance of several cancer types [De Falco et al., 2005; De Falco et al., 2008; Giacinti et al., 2008; Kikuchi et al., 2010; Romano and Giordano, 2008; Zhou and Zhou, 2010]. However, the role of CDK9 in distinct pathologies differs accordingly to the specific tissue pathological progression of the disease. In this chapter, we are going to describe them, reporting the list of the main pathologies in which CDK9 is involved (Table 1).

Table 1. Summary of the main studies that have linked CDK9 to oncogenesis, and other pathologies.

3.1 CDK9 AND CANCERS

CDK9 has a part in numerous types of cancers through the BRD4-dependent recruitment of p-TEFb for the transcription of the MYC gene, a downstream proto-oncogene involved in cell growth and cell cycle progression [Lu et al., 2015]. Also, the increased effect of Mcl-1 by over-stimulatory

effect of CDK9 is one of the mechanisms proposed that leads to cell survival and subsequent cancer development (Table 2) [Yin et al., 2014].

Table 2. CDK9 direct and indirect interacting molecules in pathological pathways.

As mentioned before, p-TEFb has proven participation in the development of several pathological processes. However, through which mechanisms does all of this occur? P-TEFb directly activates p53, a tumor suppressor protein that stops the cell cycle in the presence of any genetic abnormality, and inactivates the retinoblastoma protein (pRb), which is a pathway through which CDK9 might cause cell growth abnormalities [Simone et al., 2002]. The absence, or a diminished expression of CDK9, reduces the p53 protein activity and allows damaged cells to continue in the cell cycle [Albert et al., 2016]. Moreover, a decreased p53 expression increases the transcription of the Nuclear Protein Ataxia-Telangiectasia locus (NPAT), an important protein that associates with p-TEFb for histone gene expression. This process is fundamental for the activation of the DNA repair mechanism, a process that, if altered, favors the progression of damaged cells through cell cycle [Pirngruber and Johnsen, 2010].

P-TEFb participates in the normal hematopoietic and lymphoid tissue developments, as well as in their malignant turnover (Table 1). In literature, it is reported that there is an increased amount of CDK9/CyclinT1 in B and T cell precursors, in centroblasts and lymphocytes located in the interfollicular area of lymphoid tissue, as well as in memory B cells and activated CD25+, effector CD27+ and memory CD45+ T cells [Bellan et al., 2004]. Tumor Necrosis Factor (TNF) and interleukin-6 (IL-6) stimulatory signal are two other molecules proposed to be involved in the cells development mechanism induced by the overexpression of CDK9 [Bellan et al., 2004]. A more recognized pathway with CDK9 is that of hematopoietic cancers, like leukemias. The Mixed

Lineage Leukemia (MLL) gene suffers a balanced translocation in several types of leukemias (ALL, AML, MLL) and fuses to more than 50 different possible *loci* responsible for synthesis of transcription activators, mainly AF4 and ENL family of proteins. This later family of proteins is part of a multiprotein complex called Elongation Assisting Proteins (EAP) along with p-TEFb and functions as transcription activator in hematopoietic tissue [Esposito et al., 2011]. The MLL chromosomal rearrangement creates more than 50 different MLL-EAP protein complexes that prevent normal dissociation of the EAP complex from loci critical for hematopoietic cell precursors differentiation (Table 3) [Mueller et al., 2009]. Moreover, there is an increased intracellular amount of p-TEFb activity in pathological conditions, like diverse lymphomas and Hodgkins Disease [Bellan et al., 2004; De Falco et al., 2008].

Table 3. CDK9 direct and indirect interacting molecules in normal cellular pathways.

Breast cancer has a diverse genetic background that includes an altered CDK9 expression and interaction with proto-oncogenes [Mitra et al., 2016]. In breast cancer, although there is a diverse molecular heterogeneity, some of those molecular pathways are related to CDK9 expression. A recent study found an association of miRNAs, small non-coding RNAs involved in transcription and mRNA degradation, in particular miR-874 that directly targets 3'UTR of CDK9 mRNA and suppresses its expression, with breast cancer growth. It has been hypothesized that CDK9 downregulation can lead to tumor cell growth and its overexpression can activate a potential pathway for apoptosis induction of cancerous cells [Wang et al., 2014]. The overexpression of the proto-oncogene *MYB*, a transcription factor that associates with p-TEFb for transcription, is also related with poor prognosis in breast cancer patients due to its necessary role in Estrogen Receptor alpha positive (ERα+) cells proliferation [Mitra et al., 2016]. The role of CDK9 in breast cancer

has being reinforced by *in vitro* studies with novel CDK9 inhibitors, such as P276-00, SNS-032, CDKi, and CAN 508, showing increase apoptosis of tumor cells and decrease cell growth [Mitra et al., 2016].

Another scenario is that of prostate cancer, which shows a more novel role of CDK9 [Mohapatra et al., 2009]. The growth of prostatic cells requires action from androgens or steroid hormones through the induction of Androgen Receptors (AR) in the cytoplasm of prostatic cells. For several years, few publications have shown the interaction of AR with CDK9, demonstrating its role in prostatic cells by hyperphosphorylating Ser81 of AR, which is a required phosphorylated amynoacid for AR-dependent cell growth into malignant growth [Gordon et al., 2010]. This effect was confirmed with HEXIM-1 *in vitro* effects, where its overexpression is characteristic of aggressive prostatic cancer [Mascareno et al., 2012]. Prostate cancer has little scientific evidence of CDK9 malfunction and contribution to androgen receptor-dependent cancer growth [Gordon et al., 2010].

In the liver, the mostly represented form of CDK9 is $CDK9_{55}$. The concentration of $CDK9_{42}$ increases when cells progress through the cell cycle, becoming constantly predominant afterward [Shore et al., 2005]. These changes suggest that the concentration ratio of $CDK9_{42}$/ $CDK9_{55}$ is important to regulate the hepatocyte maturation. Furthermore, it has been demonstrated that CDK9 is involved in the progression of normal hepatic tissue to hepatocellular carcinoma (HCC, Table 1). HCC cells show overexpression of MYC, an oncoprotein that sustains malignancy by increasing p-TEFb activity in specific promoters responsible for malignant cells growth, altering the ratio of $CDK9_{42}$/ $CDK9_{55}$ by making $CDK9_{55}$ isoform predominant [Huang et al., 2014].

Scientific literature also supports the use of new antiCDK9 treatments against colon, cervix, lung and primary peritoneal cancer. Many studies confirm that there is a direct relationship of tumor development in these organs and CDK9. For instance, during cervical cancer progression, there is

an increased intracellular concentration of CDK9 from preinvasive lesions (comprising of CIN-I, CIN-II and CIN-III) to Squamous Cell Carcinoma [Ramdass et al., 2007]. *In vitro* models of cervical, ovarian and lung cancer and one *in vivo* model of ovarian and primary peritoneal cancer have demonstrated a response to anti-CDK9 therapy by using novel drugs, and by combining them with traditional chemotherapeutic agents (ej. Cisplatin) [Lemke et al., 2014]. Moreover, *in vitro* models of Colon Cancer and Multiple intestinal neoplasia (Min) show an antiproliferative response to novel CDK inhibitors, suggesting a potential chemopreventive role [Shao et al., 2013].

3.2 CDK9 AND OTHER DISEASES

Other than cancer, CDK9 is also involved in the pathogenesis of other diseases. In particular, research strongly supports the importance of CDK9 activity in cardiac cells and the process by which CDK9 is thought to be involved in cardiomyocytes growth is very well studied [Kikuchi et al., 2010; Matrone et al., 2015]. GATA4 is a transcription factor gene involved in embryogenesis of cardiac muscle, specially normal pattering and vascularization of cardiac cells. During heart development, GATA4 forms a complex with p300, and CDK9 interacts with these two molecules to phosphorylate p300. This is required for DNA binding and transcription activation [Sunagawa et al., 2016]. Therefore, CDK9 it's a key regulator in the transcriptional pathway during hypertrophic responses in cardiomyocytes [Sunagawa et al., 2016]: CDK9 concentration increases progressively, and heart mass size increases proportionally.

Developmental studies conducted in mice and zebrafish report that the activation of CDK9, or an increased production of cyclin T1, causes myocyte enlargement. When this occurs in adult life, it correlates with cardiac hypertrophy in mouse models, and with dilated cardiomyopathy in humans (Table 1) [Kikuchi et al., 2010].

A modest pharmacologic inhibition of CDK9 during the heart embryological development reduces ventricle size and ejection fraction, producing a string-like appearance. Moreover, it significantly reduces the number of mitotic cardiomyocytes [Matrone et al., 2015] suggesting that the therapy with CDK9 inhibitors could be considered as a potential approach for treatment of cardiovascular diseases.

CDK9 roles are important during early embryological phases of neuronal differentiation: it guarantees the embryo survival during the 2 cells stage and it cooperates during the process of formation of the neural crest components [Hatch et al., 2016]. It is also well demonstrate that expression levels of CDK9 and Cyclin T1 increase during neuron differentiation induced by retinoic acid [De Falco et al., 2005] and there is an increase of the complex activity during neuroblastoma growth. In fact, in neuroblastoma and PNET (Primary Neuroectodermal Tumor), the expression level of CDK9 increases as much as the tumor is differentiated [Turano et al., 2006]. CDK9 is also important for the differentiation of skeletal muscle cells and adypocytes (Table 1) [De Falco et al., 2000]. Satellite cells increase transcription of $CDK9_{55}$/CyclinT2a and stimulate muscle regulatory factor (MyoD) leading to an increase in myoblast population, which also allows the activation of the pathway for the development of rhabdomyosarcoma [De Falco et al., 2000; Giacinti et al., 2008; Simone and Giordano, 2007]. Specifically for this process, $CDK9_{55}$/Cyclin T1 stimulates transcription of the Peroxisome Proliferator Activated Receptor gamma (PPARγ), a receptor involved in adipocytes differentiation, which supports adipose tissue generation (Table 3) [Zhou and Zhou, 2010].

3.3 CDK9 AND VIRUSES

P-TEFb and its molecules so far have shown involvement in transcription of many genes, but the association of this to the HIV virus replication in cells has caught the eye of several researchers.

Transcription of HIV-1 is predominantly related to Tat, a viral transactivator protein that binds to Cyclin T1 (CycT1) of the p-TEFb complex [Zaborowska et al., 2016]. This interaction allows Tat to locate the p-TEFb complex at the promoter region of the nascent viral mRNA and stimulate the transcription elongation of the proviral DNA of the HIV [O'Brien et al., 2012]. The expression of Tat is proportional to the amount of HIV virus present and the association of CDK9 to the 7SKsnRNP suggests a possible mechanism for viral latency [Zaborowska et al., 2016].

Other viruses also depend on the p-TEFb complex for replication and viral latency like Human T-lymphotropic virus (HTLV-1), Herpes simplex virus (HSV-1 and HSV-2), Human cytomegalovirus (CMV), Epstein–Barr virus (EBV), Human adenovirus, Influenza A virus, Dengue virus and Kaposi's sarcoma-associated virus (KSHV) [Zaborowska et al., 2016].

The molecules involved in the viral replication and inflammatory response are summarized in Table 2 and 3, respectively.

4. CONCLUSIONS

Since its discovery more than 20 years ago, CDK9 has shown to be a key player in several physiological and pathological pathways. Through binding to Cyclin T1 and forming p-TEFb complex, this enzyme is able to phosphorylate RNA pol II on its CTD and regulate transcription elongation of most genes; control the maturation of mRNA molecules by recruiting capping enzymes and the splicing complex; regulate several different unstudied aspects of the embryological development of eukaryotic organisms; form complexes with other proteins (EAP, SEC) to adjust to tissue specific needs; and, being part of major pathways responsible of the development of highly prevalent pathologies, like Cancer and HIV.

It is fair to say that its involvement in transcription elongation makes CDK9 such an important protein and the point-of-start for other cellular functions. By controlling transcription,

subsequently the final “mold” for proteins (mRNA), CDK9 is able to influence almost all cellular functions. Herein, we summarize the different normal and pathological process CDK9 is involved in, as well as the direct and indirect molecules involved in each one, including major cancer types and viral replication mechanism.

This large array of proteins that interact with CDK9 along different cellular pathways is only the beginning in the understanding of a higher-order mechanism responsible for the development of diseases. It is of great importance to continue the research efforts in this field and, in the future, be able to improve the life of cancer patients.

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6. DISCLOSURE STATEMENT

The authors confirm that the content in this article presents no conflict of interest.

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