



Transworld Research Network  
37/661 (2), Fort P.O.  
Trivandrum-695 023  
Kerala, India

Emerging Signaling Pathways in Tumor Biology, 2010: 117-133  
ISBN: 978-81-7895-477-6 Editor: Pedro A. Lazo

## 6. Polo-like kinase 3 in normal and tumor biology

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**Abstract.** Plk3 is a member of the Polo-like kinase family. It has multiple biological functions including the regulation of cell cycle progression, cell cycle checkpoint control, cellular response to hypoxia, and apoptosis. Deregulated Plk3 is also strongly implicated in tumorigenesis. Aberrant expression of Plk3 is detected in tumors of various origins. Plk3 knockout mice develop tumors in multiple organs at an advanced age. In the past, much of the attention regarding the role of Plks in tumor biology has been primarily directed toward Plk1. The importance of Plk3 in tumor biology and its value as a target for therapeutic intervention has not yet been fully appreciated. Recent progress on Plk3 research has revealed new roles that may lead to better understanding of this elusive protein kinase in regulating stress responses and suppressing tumorigenesis.

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## Introduction

Serine/threonine (Ser/Thr) protein kinases play a central role in tumor biology. Deregulated kinase activities and/or their expression frequently lead to perturbation in cell growth control and differentiation, resulting in tumor formation. A prominent example is B-Raf kinase, whose oncogenic mutations contribute to the development of numerous human malignancies [1]. As deregulated kinase activities are often the main reason for their oncogenicity, small chemical compounds have been developed as potential anticancer drugs that target the kinase activities of various Ser/Thr protein kinases [2]. On the other hand, regulatory domains of protein kinases have also been explored as anti-proliferative or anticancer drugs [2]. Given the sheer number of genes encoding unique Ser/Thr protein kinases in the mammalian genome and the necessity of developing target-specific chemical compounds for cancer therapies, it is imperative for us to understand the biochemical and biological properties of various protein kinases in growth control and their deregulation in tumorigenesis.

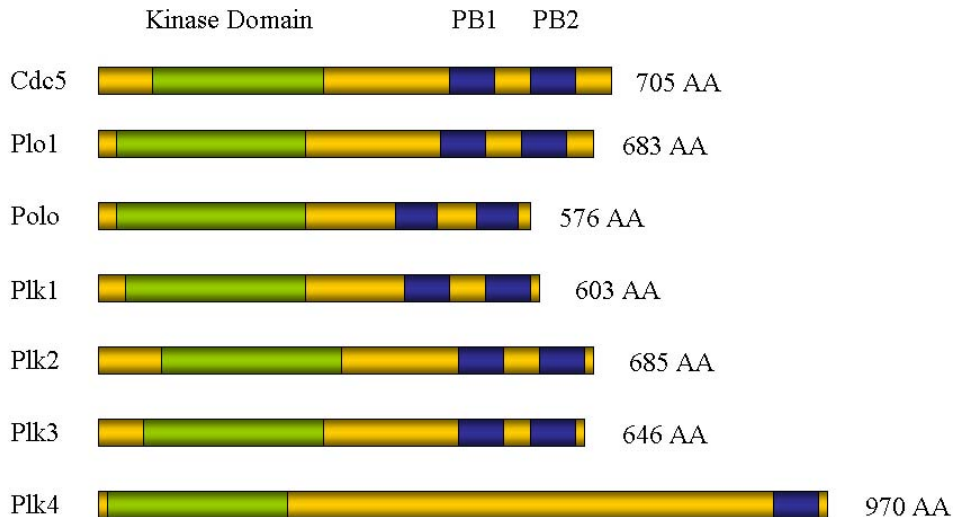
Polo-like kinases (Plks) are a family of Ser/Thr protein kinases that, among other functions, regulate cell cycle progression, apoptosis, and stress responses [3-6]. These kinases, when deregulated, have been strongly implicated in tumor initiation and development [7, 8]. Therefore there is considerable interest in exploring these kinases as targets for cancer drug development [9] although the major efforts have been devoted primary to Plk1 [10, 11]. Compared with that of Plk1, the biological role of Plk3 is much less clear. In this chapter, we attempt to summarize the work on characterization of Plk3 and discuss its significance in suppressing genomic instability and tumor angiogenesis.

## Polo-like kinases

Polo-like kinases were named after Polo, a fruit fly (*Drosophila melanogaster*) Ser/Thr protein kinase that is involved in mitosis and meiosis [12]. Polo was originally discovered from a *polo* mutation that is associated with mitotic and meiosis defects in fruit fly [13]. It was subsequently found to be a highly conserved kinase [14]. Polo thus serves as the founding member of a family of evolutionarily conserved Ser/Thr protein kinases. Polo is an essential component of the cell cycle machinery and its kinase activity and expression level are tightly regulated during the cell cycle [3, 15]. Mutations of the *polo* gene lead to abnormal mitosis and meiosis [3].

The counterparts of Polo kinase in the budding yeast and the fission yeast are CDC5 and Plo1, respectively [16]. As with the fruit fly, these proteins have a pivotal role in the mitotic stage of the cell cycle [16]. In mammals, the

Plk kinase family consists of four members including Plk1, Plk2, Plk3, and Plk4. These proteins all share significant sequence homology to Polo [17]. Structurally, all Plks are comprised of a highly conserved kinase domain at the amino-terminus and a Polo box domain (PBD) at the carboxyl-terminus [5, 17] (Fig. 1). PBD is critical for the kinase subcellular localization as well as the substrate recognition by Plks [5]. The biological functions of mammalian Plks, however, are more diverse than that of their counterparts in lower eukaryotes. Plk1 is the most studied and its functions are more in line with those of Polo in the regulation of mitosis [6]. Plk1 expression peaks at G<sub>2</sub>/M phases, consistent with its role in mitosis [18]. The functions of Plk2, Plk3, and Plk4, however, are much less understood. Available evidence indicates that these proteins may have more diverse functions than that of Plk1 [6]. This notion is reinforced by findings revealing that these proteins have expression patterns quite different from those of Plk1 during the cell cycle [18]. In addition to the potential role in mitosis, Plk2, Plk3, and Plk4 appear to have functions in regulating the G<sub>1</sub>/S transition, S phase progression [19, 20], centrosome dynamics, stress responses [20-24], and/or synaptic activities [25]. Plks, or their deregulated activities, have also been strongly implicated in tumor development, especially Plk1, Plk3 and Plk4 [7, 8, 26, 27].



**Figure 1.** Basic Structures of Plk Family Members.

### Polo-like kinase 3 (Plk3)

Mouse Plk3 was initially identified as a fibroblast growth factor-inducible kinase (Fnk) from NIH 3T3 cells in a differential display analysis [28]. The human counterpart was independently isolated and named Prk as a proliferation related kinase [29]. The amino acid sequence of human Plk3

shares 36% and 33% overall identity to Plk1 and Polo, respectively [29]. Plk3 has the structure of a typical Plk family member, with an N-terminal kinase domain and a C-terminal PBD with two Polo boxes [17] (Figure 1).

Plk3 is considered an immediate early response gene, whose expression does not depend on *de novo* protein synthesis [18, 28, 29]. Its mRNA expression level reaches the maximal level one hour after stimulation with fibroblast growth factor or serum addition to the serum-starved NIH 3T3 cells and returns to the basal level 8 hours post-stimulation [18, 28, 30]. Plk3 protein level is fairly constant in mitogen-stimulated cells throughout the cell cycle [18, 21, 30]. A recent report, however, showing that Plk3 protein level is also cell cycle-regulated with a pattern similar to that of its mRNA, peaking at the G<sub>1</sub> phase [19]. The kinase activity of Plk3 also oscillates during the cell cycle [31]. Immunocomplex kinase assays show that whereas the Plk3 kinase activity is low in G<sub>1</sub> and G<sub>1</sub>/S phases, it dramatically increases during late S/G<sub>2</sub> and remains at a moderate level at M phase [31]. Plk3 activity is also subjected to regulation by a variety of stress conditions [21, 23, 24, 32, 33].

## Plk3 and tumorigenesis

Tumorigenesis is a cellular transformation process that causes uncontrolled cell division and proliferation, resulting in a disease phenotype in multi-cellular organisms. Perturbations of many biological processes that control cell proliferation, apoptosis, stress responses, and angiogenesis have the potential to trigger malignant transformation [34].

The association of Plk3 with tumor development was first revealed when the human Plk3 (Prk) was examined in human primary tumor samples [29]. In contrast to Plk1, whose activity and expression are generally positively correlated with tumor formation, Plk3 activity or expression is often negatively correlated with tumor development. Thus, Plk3 is commonly regarded as a tumor suppressor [7, 8]. This notion has been confirmed by a mouse genetic study [22]. Compared with wild-type littermates, Plk3 null mice are prone to the development of malignancies in several organs [22].

Through analysis of more than a dozen of lung cancer patient samples, Plk3 mRNA was found to be significantly downregulated in a majority of lung carcinoma samples [29]. This abnormal mRNA expression appears to be a result of reduced *PLK3* mRNA transcription [29], suggesting that reduced Plk3 expression may be associated with tumor development. This notion is supported by the observation that although three polymorphisms were identified from 40 lung tumor cell lines, no missense or nonsense mutations were found in Plk3 [35]. Thus, mutations of Plk3 coding sequences appear to be rare in lung cancer.

Reduced expression of *PLK3* also occurs in human head and neck cancer [36]. In a survey of primary head and neck squamous-cell carcinomas (HNSCC) from 35 patients, 26 samples have reduced *PLK3* mRNA levels compared with corresponding normal tissues. Two samples have an undetectable level of *PLK3* expression [36]. This connection of *PLK3* expression with head and neck cancer is reinforced by the finding that the *PLK3* gene is located at chromosome 8p21 where high frequency of loss of heterozygosity happens in many human cancers [36-38].

Plk3 is also implicated in the development of colon cancer [39]. Expression of *PLK3* mRNA is significantly lower in rat colon tumors that have been induced by azoxymethane than it is in the corresponding normal tissues [39]. Moreover, *PLK3* mRNA levels in rat colon tumors appear to be influenced by diet since tumors isolated from rats fed high fat diet supplemented with fish oil exhibit less reduction in *PLK3* expression than those isolated from rats fed the same diet supplemented with corn oil [39].

A positive correlation between Plk3 expression/activity and cancer formation has also been reported. An immunohistochemical study shows that Plk3 is expressed at a moderately high level in cystadenomas [40]. More than 5% of ovarian carcinomas are Plk3-positive whereas Plk3 expression was low in normal ovarian surface epithelium and borderline tumors [40]. In addition, LFM-A13, an inhibitor of Plks with a significant inhibitory activity toward Plk3, is able to delay progression of MMTV/neu transgenic mouse model of HER2 positive breast cancer [41].

## **Plk3 and cell cycle progression**

### **Plk3 in regulating G<sub>2</sub>/M phases**

Plk3 is involved in regulating multiple phases of the cell cycle. In most cells, Plk3 is predominantly located around the nuclear membrane in interphase of the cell cycle [42]. It concentrates at the centrosomes in a microtubule-dependent manner [42]. During mitosis, Plk3 is closely associated with spindle poles and mitotic spindles [42]. A significant amount of Plk3 can also be found in midbody in telophase [42]. Plk3 overexpression induces rapid cell cycle arrest at the M phase followed by apoptosis, likely as a result deregulation of microtubule dynamics and centrosomal function [42]. The subcellular localization of Plk3 to the centrosomes, spindle poles and the midbody is driven by the PBD domain of the kinase [43]. Both Polo boxes of Plk3 are required for its localization [43]. Interestingly, overexpression of the PBD domain alone can cause significant cell cycle arrest and abnormal cytokinesis [43]. This is consistent with another report that overexpression of

Plk3 inhibits cell proliferation and colony formation as a result of incomplete cytokinesis [44].

The functional conservation of Plk3 is clearly illustrated by two lines of evidence. Human *PLK3* transcripts greatly enhance progesterone-induced meiotic maturation of *Xenopus oocytes*, whereas the corresponding antisense *PLK3* transcripts inhibit the maturation process [31]. In *S. cerevisiae*, ectopic expression of human Plk3 was able to rescue the temperature-sensitive phenotype of a CDC5 (the yeast Plk homolog) deficient strain of the budding yeast [31]. These studies thus strongly suggest that Plk3 may also regulate the onset and/or progression of mitosis and meiosis.

Plk3 phosphorylates and regulates the subcellular localization of Cdc25C phosphatase [45, 46], a key regulator of the G<sub>2</sub>/M transition by dephosphorylating inhibitory phosphorylation sites of CDK1 thereby activating the master regulator of mitosis [47]. Plk3 directly interacts with Cdc25C and phosphorylates it *in vitro* at Ser216 [45], a residue critical for binding to scaffold protein 14-3-3 when phosphorylated [48, 49]. Binding of Cdc25C to 14-3-3 sequesters Cdc25C to the cytosol, thereby effectively inhibiting its function in the nuclei [50]. Plk3 is also found to phosphorylate Ser191 and Ser198 of the Cdc25C [46]. Similar to Ser216, phosphorylation of these two residues also promotes accumulation of Cdc25C in the nuclei [46]. Thus, serine to alanine mutation of Ser191 prevents the nuclear accumulation [46]. Consistent with this observation, ectopically expressed Plk3 promotes the accumulation of Cdc25C protein in the nuclei whereas Plk3 knockdown with RNA interference inhibits its nuclear accumulation [46]. Together, these findings indicate that Plk3 is an important regulator of the cell cycle at the G<sub>2</sub>/M transition.

### **Plk3 in regulating G<sub>1</sub>/S phases**

Plk3 also mediates the G<sub>1</sub>/S transition and is required for the S phase entry [19, 51]. Plk3 protein levels are regulated during the cell cycle that peak at the G<sub>1</sub> phase [19, 51], consistent with the expression pattern of Plk3 mRNA [18, 28, 30]. Knockdown of Plk3 by transfecting siRNA prevents cells from entering the S phase of the cell cycle [19]. Mechanistically, Plk3 appears to be required for cyclin E expression since Plk3 depletion significantly reduces cyclin E levels [19]. Regulation of cyclin E by Plk3 appears to be mediated through a posttranscriptional mechanism [19]. It has been proposed that Plk3 may regulate cyclin E levels by modulating the activity of phosphatase Cdc25A [19], which is a potential target of Plk3 [52]. Cdc25A is known to regulate the activity of the cyclin E/Cdk2 complex [53, 54].

Plk3 also phosphorylates proteins that are important for regulating DNA replication [20, 55]. Plk3 but not Plk1 can phosphorylate topoisomerase II $\alpha$  at Thr1342 [55]. Topoisomerase II $\alpha$  has an essential role in DNA replication/transcription, cell cycle regulation, cell proliferation, and cancer development [56]. Although the functional significance of Thr1342 phosphorylation remains poorly understood, this finding may reveal a potential new mechanism by which cell proliferation and tumor formation is differentially regulated by Plk3 and Plk1 [55]. Moreover, Plk3 interacts with and phosphorylates p125, the major subunit of DNA polymerase  $\delta$  (Pol  $\delta$ ), and the phosphorylation occurs on Ser60 [20]. The significance of the physical interaction and phosphorylation of Pol  $\delta$  by Plk3 remains to be elucidated. It is speculated that the phosphorylation may regulate the subcellular localization of Pol  $\delta$  because the region of p125 contains a nuclear localization signal.

### **Plk3 in Golgi fragmentation**

Fragmentation of the Golgi apparatus in mammalian cells is an essential step in mitosis entry. It is a process in which Golgi stacks are reversibly disassembled into small vesicles and tubules during mitosis to ensure correct partitioning of the Golgi content between daughter cells [57]. Several lines of evidence show that Plk3 is involved in the Golgi fragmentation process [58-60]. Plk3 is found to localize at the Golgi apparatus in interphase of the cell cycle; it disintegrates and redistributes in a manner similar to that of Golgi stacks [59]. Nocodazole, a drug that disrupts microtubules and induces mitotic arrest and Golgi fragmentation, activates Plk3 [59, 60]. Plk3 interacts with Golgi-specific protein giantin; a Golgi-specific poison brefeldin A disperses Plk3 signals in a manner similar to that of the Golgi apparatus [59]. Ectopic expression of wild-type but not the kinase-defective mutant of Plk3 leads to marked Golgi breakdown [59, 60]. Subsequent studies show that MEK1, a MAP kinase kinase known to mediate Golgi fragmentation, interacts and activates Plk3 [60]. Plk3 also colocalizes with phosphorylated MEK1 at the spindle poles [60]. Activation of Plk3 by nocodazole and Golgi fragmentation induced by Plk3 can be blocked by specific MEK1 inhibitors [60]. These results indicate that Plk3 is an essential regulator of Golgi fragmentation during mitosis and that MEK1 is an upstream activator of Plk3 in mediating this process.

A recent study identifies vaccinia-related kinase1 (VRK1) to be the direct downstream target of Plk3 in regulating Golgi fragmentation [58]. VRK1 interacts and colocalizes with the Golgi complex and Plk3 throughout mitosis [58]. Plk3 phosphorylates VRK1 at Ser342 of the C-terminal region and this phosphorylation is necessary for VRK1 to mediate Golgi fragmentation.

Thus, a serine-to-alanine point mutation abolishes the capability of VRK1 to promote Golgi fragmentation induced by Plk3 or MEK1 [58]. Furthermore, both VRK1 kinase activity and phosphorylation by Plk3 at Ser342 are necessary for its function in Golgi fragmentation. Moreover, knockdown of VRK1 by siRNA or overexpression of kinase-dead VRK1 also inhibits Plk3 or MEK1-induced Golgi fragmentation [58]. Combined, available evidence has revealed the MEK1→Plk3→VRK1 signaling axis in the regulation of Golgi fragmentation in mitosis.

### **Plk3 in regulating apoptosis**

Apoptosis or programmed cell death is a controlled suicidal process that is vitally important in maintaining homeostasis of tissues in multicellular organisms [61]. Apoptosis serves as a tumor suppressing mechanism by which old, abnormal, and/or excessive cells in tissues are removed [34, 62, 63]. Dysregulated apoptosis often contributes to tumor development [34, 63].

The role of Plk3 in apoptosis was initially revealed in the study of ectopically expressed Plk3 [32, 44]. Ectopic expression of Plk3 causes cell cycle arrest followed by chromatin condensation and apoptosis of cultured cells [32, 42, 44]. Plk3 appears to mediate apoptosis through a kinase-dependent and a kinase-independent mechanism [42, 64]. It is also shown to induce apoptosis in a biphasic manner: a rapid apoptosis that requires Plk3 kinase activity and p53, and a delayed onset apoptosis that is independent of Plk3 kinase activity and p53 [64]. Consistently, kinase defective Plk3 is less capable of inducing apoptosis compared to wild type Plk3 [42, 64].

Plk3 promotes the kinase-independent apoptotic function mainly through its C-terminal Polo box domains because ectopic expression of the Polo box domains alone is sufficient to induce apoptosis [32, 43, 44]. However, another study has identified the first 26 amino acids at the N-terminus of Plk3 to be essential for induction of apoptosis when it is overexpressed [64]. DNA damage- or superoxide-induced apoptosis requires the kinase activity of Plk3 [32, 64]. Plk3 phosphorylates tumor suppressor protein p53 at Ser20 in response to these stimuli and induces apoptosis through the p53-mediated proapoptotic pathway [32, 64]. Moreover, Plk3 can induce apoptosis through disrupting microtubule integrity [42].

Plk3 has been identified as a key component in NF-κB-dependent apoptosis [64]. A κB enhancer element is identified at the promoter region of *PLK3* gene [64]. This element is responsible for NF-κB mediated Plk3 expression in response to a panel of known NF-κB activators, including chemotherapeutic drug Doxycycline [64]. Plk3 appears to play an essential role in Doxycycline-induced apoptosis, since knockdown of Plk3 expression

with siRNA dramatically inhibits Doxycycline-induced apoptosis [64]. Increase of Plk3 levels alone does not seem sufficient for apoptosis because TNF- $\alpha$  strongly activates Plk3 expression but fails to induce apoptosis [64]. Thus, Plk3 is necessary but not sufficient for NF- $\kappa$ B-mediated apoptosis.

## **Plk3 in stress responses**

### **Plk3 in DNA damage response**

Genotoxic stresses such as ultraviolet light and ionizing radiation generate reactive oxygen species (ROS). ROS induces DNA damages and mutations that lead to genomic instability, thereby promoting tumor formation and progression [65, 66]. ROS are known to regulate DNA damage responses through the activation of p53, which leads to cell cycle arrest, apoptosis or replicative senescence [67]. ATM (mutated in ataxia telangiectasia) functions as an upstream regulator of p53 that is activated by oxidative stresses [68]. ATM regulates phosphorylation of p53 directly or indirectly and is thought to be a sensor for oxidative stresses [68].

Plk3 is activated by and mediates cellular responses to DNA damage that produces ROS [21, 23, 32, 69, 70]. Genotoxic agents such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), ionizing radiation, methylmethane sulfonate, ultraviolet light, and adriamycin strongly activate Plk3 [21, 32, 69, 70]. This is different from Plk1 whose activity is inhibited by these agents [32]. Plk3 mRNA is also inducible by ionizing radiation [33]. Plk3 activation induced by genotoxic agents is ATM-dependent [21, 32, 69]. Thus, H<sub>2</sub>O<sub>2</sub> and ionizing radiation mimetics are unable to activate Plk3 in ATM-deficient cells [21, 69]. Furthermore, inhibition of ATM using caffeine blocks adriamycin- or ionizing radiation-induced Plk3 activation [21, 32]. Plk3 is phosphorylated when treated with ionizing radiation and this phosphorylation is also ATM-dependent [21]. Further experimentation, however, fails to detect direct phosphorylation of Plk3 by ATM, indicating that ATM may promote Plk3 phosphorylation and activation through another kinase(s) [21]. One of the candidate kinases that phosphorylates Plk3 appears to be Chk 2, which is known to be activated by ATM in response to genotoxic stresses [21].

The downstream effector of Plk3 in mediating DNA damage responses is p53 [21, 32, 69]. Plk3 is able to phosphorylate p53 in response to DNA damage [21, 32, 69]. Activation of Plk3 by H<sub>2</sub>O<sub>2</sub> is accompanied by phosphorylation of p53 at Ser9, Ser15, and Ser20 residues [69]. Phosphorylation of p53 at these residues in response to H<sub>2</sub>O<sub>2</sub> correlates with its transcriptional activity since the level of p21, whose expression is positively regulated by p53, is also elevated [69]. Further analysis reveals that

phosphorylation of Ser20, but not other residues, after H<sub>2</sub>O<sub>2</sub> treatment is dependent on Plk3 [69]. Furthermore, H<sub>2</sub>O<sub>2</sub> fails to induce Ser20 phosphorylation of p53 in a Plk3-deficient cell line; the kinase-defective Plk3 suppresses H<sub>2</sub>O<sub>2</sub>-induced Ser20 phosphorylation [69]. Phosphorylation of Ser20 of p53 is confirmed in an *in vitro* assay using recombinant Plk3 and p53 proteins [32]. Ser20 of p53 is also a target of protein kinases Chk1 and Chk2 that are downstream components of ATM and are essential for DNA damage checkpoint control [71, 72]. Plk3 is thus proposed to work in parallel with Chk1 and Chk2 to reinforce DNA damage checkpoint responses or to preferentially mediate certain types of DNA damage insults [32]. Plk3 is also found to directly regulate Chk2 and vice versa [21, 70]. Chk2 interacts directly with and activates Plk3 both *in vitro* and *in vivo*; this interaction is enhanced after DNA damage [70]. Intriguingly, Plk3 also phosphorylates Chk2 and contributes to its full activation [21]. Collectively, these results underscore the dynamic role of Plk3 in the DNA damage checkpoint control.

### Plk3 in hypoxic stress responses

Tumor progression and metastasis require blood supply to provide oxygen and nutrients to the newly formed tumor masses. The formation of new blood vessels or angiogenesis is therefore essential for tumor growth [73, 74]. Cellular responses to hypoxia play an important role in tumor growth since hypoxia triggers tumor angiogenesis [75]. Hypoxia-inducible factors (HIFs) are the key sensors and regulators of hypoxic responses, which, upon induction by hypoxia, promote expression of multiple genes essential for mediating angiogenesis. Among the HIF-activated gene products are: fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and glucose transporters [75].

Plk3 can be activated by hypoxia [24]. Hypoxia-activated Plk3 increases the activity of AP-1 transcription factors and promotes apoptosis [24]. A recent study has demonstrated that Plk3 is involved in controlling HIF-1 $\alpha$  levels in response to hypoxic conditions [22]. Specifically, compared with wild-type mouse embryonic fibroblasts (MEFs), *PLK3* null MEFs exhibit a hyper-sensitive response to the treatment with hypoxia or with nickel ions and contain an elevated level of HIF-1 $\alpha$  but not of HIF-1 $\beta$  [22]. Consistently, expression of VEGF-A is also higher in *PLK3*<sup>-/-</sup> MEFs than that in wild type MEFs [22]. More importantly, *PLK3* null mice display an increased tumor incidence; tumors developed in these mice display an increased vasculature [22]. Transfection analyses also confirm the role of Plk3 in regulating the stability of HIF-1 $\alpha$  [22]. Ectopically expressed Plk3 is able to suppress the expression and nuclear accumulation of HIF-1 $\alpha$  in HeLa cells [22]. The

inhibition of HIF-1 $\alpha$  nuclear translocation appears to be dependent on its kinase activity since overexpression of Plk3 kinase domain alone is sufficient to suppress HIF-1 $\alpha$  accumulation in the nucleus under hypoxic conditions [22].

The exact mechanism by which Plk3 regulates HIF-1 $\alpha$  levels and its activities remains to be elucidated. HIF-1 $\alpha$  is a very labile protein that is rapidly degraded under normoxia via the proteasome [76]. After hydroxylation, which is an O<sub>2</sub>-sensitive process, hydroxylated HIF-1 $\alpha$  is recognized by von Hippel-Lindau factor (VHL, a ubiquitin E3 ligase) for polyubiquitination and subsequent proteasomal degradation [77]. HIF-1 $\alpha$  degradation can also be positively controlled by direct phosphorylation. GSK3 $\beta$  phosphorylates HIF-1 $\alpha$  in the oxygen-dependent degradation domain, resulting in its destabilization via a VHL-independent but proteasome-dependent process [78]. Given that HIF-1 $\alpha$  is super-induced by hypoxia or hypoxic mimetics in *PLK3* null MEFs [22], it is tempting to speculate that Plk3 may regulate HIF-1 $\alpha$  protein stability via a phosphorylation-dependent process as well.

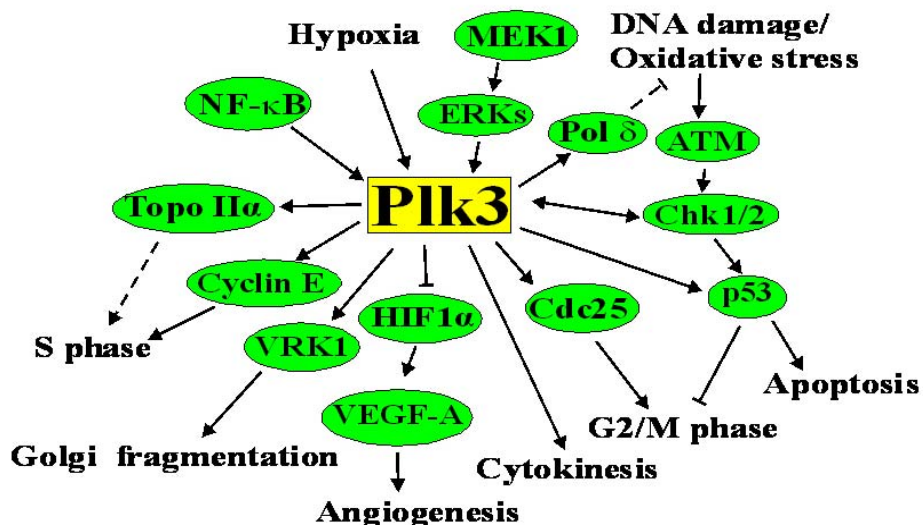


Figure 2. Plk3 Regulatory Network.

## Conclusion remarks

The relationship between Plk3 and normal or abnormal cell proliferation has been established since its initial identification. To date, interest in the role of Plk3 in tumor biology has lagged behind that of Plk1, a structurally close, and perhaps functionally-related component. Earlier work has focused primarily on the role of Plk3 in cell cycle regulation. Recent studies suggest

that Plk3 may have broader functions. The potential role of Plk3 in mediating hypoxic responses is particularly interesting since it can lead to the discovery of a new player important to the regulation of tumor angiogenesis. Despite extensive research efforts in the past decade, Plk3 remains largely an elusive protein kinase in terms of its physiological substrates and biological functions. Much needs to be done in order to obtain a clear picture on Plk3's roles in normal biology, as well as to explore it as a potential anti-tumor target. Summarized in Figure 2 is the current knowledge about molecular interactions between Plk3 and cellular gene products in the regulation of normal and abnormal biological processes.

## Acknowledgements

We thank Jeffery Winkles for his assistance in the study of *PLK3* knockout mice. We are grateful for co-workers in the laboratory for various technical support and intellectual input. We also thank Mrs. Nedda Tichi for administrative support. This work was supported in part by grants from the National Institutes of Health to WD (CA074229, CA090658).

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