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Review

Modelling the Functions of Polo-Like Kinases in Mice and Their Applications as Cancer Targets with a Special Focus on Ovarian Cancer

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Abstract: Polo-like kinases (PLKs) belong to a five-membered family of highly conserved serine/threonine kinases (PLK1-5) that play differentiated and essential roles as key mitotic kinases and cell cycle regulators and with this in proliferation and cellular growth. Besides, evidence is accumulating for complex and vital non-mitotic functions of PLKs. Dysregulation of PLKs is widely associated with tumorigenesis and by this, PLKs have gained increasing significance as attractive targets in cancer with diagnostic, prognostic and therapeutic potential. PLK1 has proved to have strong clinical relevance as it was found to be over-expressed in different cancer types and linked to poor patient prognosis. Targeting the diverse functions of PLKs (tumor suppressor, oncogenic) are currently at the center of numerous investigations in particular with the inhibition of PLK1 and PLK4, respectively in multiple cancer trials. Functions of PLKs and the effects of their inhibition have been extensively studied in cancer cell culture models but information is rare on how these drugs affect benign tissues and organs. As a step further towards clinical application as cancer targets, mouse models therefore play a central role. Modelling PLK function in animal models, e.g., by gene disruption or by treatment with small molecule PLK inhibitors offers promising possibilities to unveil the biological significance of PLKs in cancer maintenance and progression and give important information on PLKs' applicability as cancer targets. In this review we aim at summarizing the approaches of modelling PLK function in mice so far with a special glimpse on the significance of PLKs in ovarian cancer and of orthotopic cancer models used in this fatal malignancy.

Keywords: polo-like kinases; oncogenesis; cancer treatment; ovarian cancer; mouse models

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1. Polo-Like Kinases and Their Physiological Functions

The founding name *polo* deduces from the effect of the gene-knockout first described in *Drosophila melanogaster* in form of abnormal spindle poles during mitosis [1]. Its orthologue in vertebrates encodes the protein polo-like kinase 1 (*PLK1*) which proofed to act as a key regulator of the cell cycle [2]. PLK1 belongs to a family of serine/threonine protein kinases, comprising five members PLK1 to PLK5 (in order of identification) in higher eukaryotes, among which PLK1 is far the most thoroughly studied and best characterized member (as reviewed by [3–6]). Family members (with the exception of PLK5) contain an ATP-binding catalytic serine/threonine kinase domain at the amino-terminus transferring a phosphate group to a multiplicity of cellular targets. Separated from the kinase domain by a linker region are, with the exception of PLK4, two polo-box motifs folding together and representing the non-catalytic regulatory domain in the carboxy-terminus. These motifs are known as the polo-box domain which is unique to the PLK family (for reviews

see [7,8]). PLK4 is singular and the least representative family member with respect to its triple polo-box architecture including a cryptic dimeric one [9], for reviews see [10,11]. Catalytic as well as regulatory domains are evolutionary highly conserved. The polo-box domain recognizes and binds to ligands with phosphorylated serine/threonine motifs, thereby directing the enzyme to differential subcellular locations and target proteins as substrates and modulating the kinase activity [12–14], for reviews see [6,15]. PLK1, PLK2, and PLK3 share high sequence homology especially with respect to their kinase domain but to a lesser extent also to the polo-box domain. In contrast, PLK4 as well as PLK 5 reveal divergent structural properties (Figure 1).

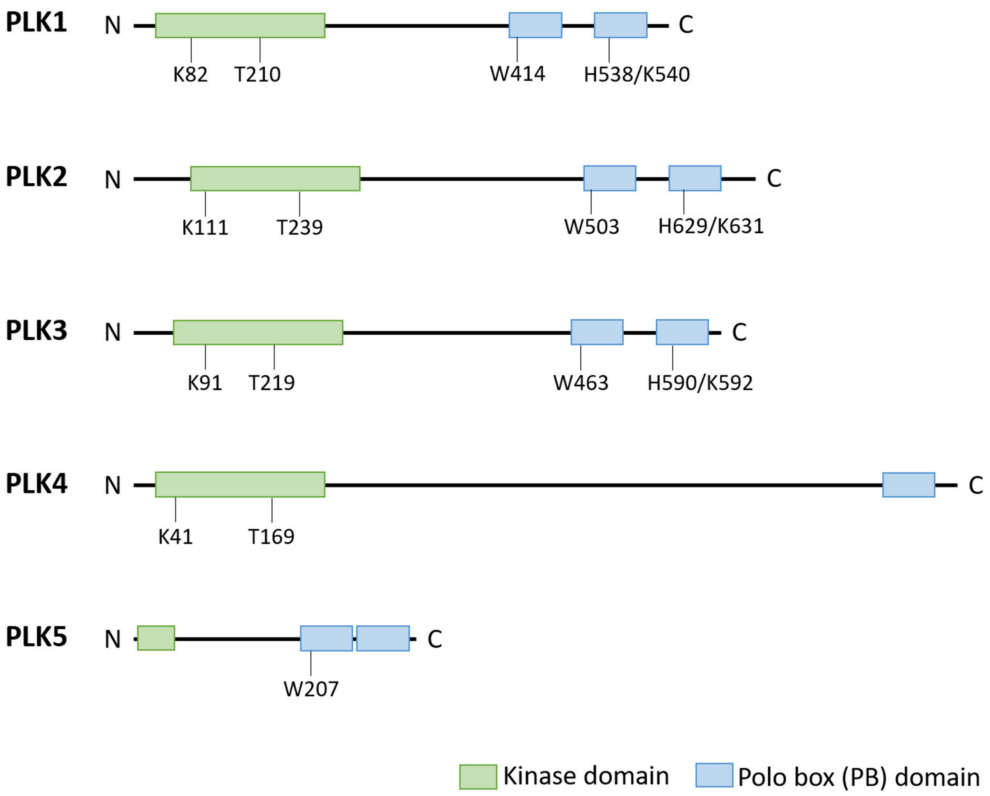

Figure 1. Structural differences between PLK family members PLK1–PLK5 [16]. PLK family members show a similar structure except PLK5. PLK1–PLK4 contain a highly conserved kinase domain at the *N*-terminal end and a non-catalytic polo box domain (PBD) at the *C*-terminus. The PBD is formed by three polo-boxes (PLK4) or two polo box motifs. These PBs are involved in substrate binding and regulation of kinase activity. Key residues of the kinase domain (acceptor lysine and T-loop threonine) and the PBs for substrate recognition are indicated. PLK5 has lost its catalytic activity in humans and expresses only a small portion of the kinase domain along with the PBD, the second PB has lost the conserved key residue involved in phosphosubstrate binding.

Family members are different in expression patterns within cells and tissues, subcellular localizations and functions and some exhibit a tissue-specific or -restricted expression. The PLK family, in particular PLK1 and PLK4, is best known for the orchestration of key events during mitosis (Figure 2) and plays important roles in cell cycle progression and regulation as well as in cell response to various types of stress (for reviews see [3,6,17,18]). Their differentially expressed members are key players in complex signaling networks controlling vital cellular functions [4,16,19,20].

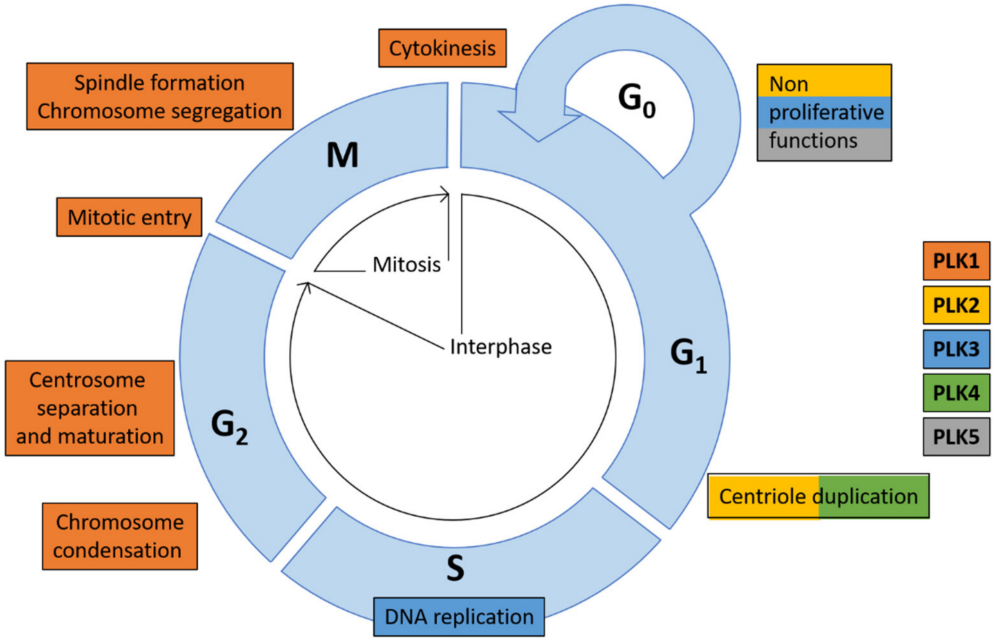

Figure 2. Functions of PLK family members in cell cycle [16]. PLK family members are essential for cell cycle processes, such as centriole duplication (PLK2 and PLK4), DNA replication (PLK3), chromosome condensation, centrosome separation and maturation, mitotic entry, spindle formation and chromosome segregation and cytokinesis (all PLK1). Besides cell cycle-related functions PLKs exert a multitude of vital cellular functions being part of complex signaling networks.

PLK family members have partial-or non-overlapping substrates and each kinase serves specific functions in a wide variety of cellular processes. PLK1 and PLK4 share high expression in actively proliferating cells and tissues predominantly during S/G2 and M-phase contrary to PLK2, PLK3, and PLK5 (Figure 2).

PLK1 (also known as PLK and STPK13) is highly expressed in embryonic as well as in adult cells and tissues with intense proliferation. It is essential for life as *Plk1*-deficient mice are embryonic lethal [21] although *Plk1* haplo-insufficient mice are viable [22]. *PLK1* is a pivotal gene for the regulation and control of cell cycle progression and is involved in almost every event of the mitotic cell division itself (for reviews see [3,5,6,8,17,23,24]). Accordingly, expression of *PLK1*/PLK1 is low during interphase and high during mitosis at both mRNA and protein level with a peak at G2/M phase transition. Expression levels remain high until mitotic exit [25]. Intracellular half-life is quite short with approx. 9 h reviewed by [26,27]. The expression is p53-dependent and follows the upregulation of the mitotic kinases Cyclin B1 and cyclin-dependent kinase 1 (CDK1) during cell division [28]. In addition, PLK1 is a central actor during meiosis [29,30] and reviewed by [20]. As a multi-functional mitotic kinase, PLK1 is localized to various subcellular locations depending on the cell cycle stage and it exerts stage-dependent specific functions throughout most of the cycle (for reviews see [6,31]). During interphase, PLK1 is associated with centrosomes as it is required for centrosomal maturation in G2 [32,33]. It participates in checkpoint recovery, the timing of mitotic entry, chromosome condensation and bipolar spindle formation [34–36]. Via stabilization of microtubule-kinetochore attachment [37–40], PLK1 ensures accurate chromosome alignment in the metaphase plate and orchestrates correct chromosomal segregation in anaphase [41,42]. Finally, PLK1 coordinates execution of cytokinesis and mitotic exit [43–45].

However, the multiplicity of substrates of PLK1 beyond mitosis (for reviews see [31,46,47]) suggests much more complex roles in cell cycle regulation and underlines vital interphase-related cellular functions far beyond the traditional mitotic ones which are controlling microtubules in the centrosomes, the spindle and the kinetochore. These non-mitotic functions include ciliogenesis, DNA replication, transcription and translation, stress signaling

and cell responses to DNA damage as well as the regulation of tumor suppressor p53 activity and the targeting of the apoptotic signaling pathway [6,17,46–51]. PLK1 is engaged in autophagy-mediating pathways involving mechanistic target of Rapamycin (mTOR) as a key kinase promoting (cancer) cell growth [52–54].

PLK2 (also known as serum inducible kinase SNK, hSNK or hPlk2) [55] is predominantly expressed in non-proliferating tissues and not associated with high mitotic rates. It is found in a rather tissue-specific manner compared to *PLK1* [19,56]. Attention has been drawn to participation in development, especially of the mammary gland epithelium [57,58] and to non-catalytic roles in the adult nervous system, where it is highly expressed and associated with the control of neuronal activity and synaptic function [59–62] repressing synaptic hyperactivity [63]. Mitosis-associated kinase activity is seen in centriole duplication, were PLK2 is localized during early G1 phase [64,65]. In addition, it plays a role in S-phase checkpoint [66]. Intracellular half-life is short (reviewed by [26]). PLK2 is involved in genotypic stress response and upregulated after DNA-damage, thereby participating in the activation of a p53-dependent G2 checkpoint and ensuring genomic stability [67].

PLK3 (also named cytokine inducible kinase CNK, FGF-inducible kinase FNK or proliferation-related kinase PRK) is a least explored family member with diverse functions found in a variety of tissues and organs (e.g., ovary, testis, skin, placenta, brain, lung, gastrointestinal mucosa, and hematopoietic tissues). *Plk3*-deficient mice develop rather normal and are fertile [68,69] which may, however, result from compensatory mechanisms. Its expression is cell cycle-regulated and peaks transiently during G1 phase. During mitosis, PLK3 is in close association with spindle poles and mitotic spindle [70]. The protein is very stable (reviewed by [26]). PLK3 is enriched at the plasmalemma and at Golgi membranes [71,72], reviewed by [73], possibly involved in the modulation of cellular adhesion and in intracellular trafficking [71]. Importantly, PLK3 participates in the Fas ligand-induced pathway of apoptosis [74], reviewed by [72]. Supposed roles for the progression of the cell cycle, namely entry into S phase are questioned [69,75] whereas PLK3 has been shown to participate in G2/M transition through interaction with cell division cycle Cdc25C protein phosphatase [76]. PLK3 has been reported to function in the mediation of cellular response including cell cycle checkpoint activation [77,78] after various forms of stress including hypoxic, DNA damage and osmotic stress [79,80], for reviews see [47,72]. Results from *Plk3*-knockout and its inactivation, however, argue against significant roles in cellular stress response [73]. The current data underline that PLK3 regulatory role in the cellular network is rather complex and diverse [72,81].

PLK4 (also known as SAK, STK18 or MCCRP2) is highly expressed in actively dividing cells during development and in adult tissue as accounts for PLK1 [19,82]. *Plk4*-deficiency leads to embryonic death [83]. Contrary to *PLK1*, its expression starts in G1-phase and gradually increases to a peak in G2-phase with differential localization according to cell cycle stage [83]. The protein is quickly degraded (reviewed by [26]). PLK4 is essential for centriole biogenesis driving centriole duplication and mitotic progression [84–87], for reviews see [6,10,11,47,88,89]. Like PLK1, PLK4 acts as an integrative protein involved in the control of cell division. It localizes to the centrosome, kinetochore, cleavage furrow and midbody during the course of the cell cycle and participates in the maintenance of chromosomal stability [90,91]. PLK4 triggers mitosis via phosphorylation of Cdc25 [92]. PLK4 is required for cytokinesis. It is a microtubule-associated protein capable of promoting de novo centrosome formation [93]. Thus, PLK4 and PLK1 are key mitosis- and cell cycle orchestrating kinases taking over separate functions with both being tightly regulated. PLK4 substrates are not restricted to centriolar-associated networks but a multiplicity of potential target substrates point to participation in non-centriolar signaling networks [94]. PLK4 interacts with a variety of proteins including p53 and nuclear factor kappa B (NFκB) affecting its expression, stability and activity (reviewed by [10,89]).

PLK5 (also known as PLK-5, PLK5P, and SgK384ps) is less explored but unique member of the family as it lacks typical kinase activity due to a truncated kinase domain [95]. *PLK5* expression is restricted to a few tissues. These are eye, male and female tissues and

mainly brain, were it is implicated in neuron differentiation, namely axonal outgrowth, and neuronal activity [96], reviewed by [6]. PLK5 localizes to the nucleolus, is downregulated in proliferating cells and ectopically expressed in response to multiple stressors, esp. DNA damage or microtubule disruption, inducing G1 cell cycle arrest and apoptotic cell death [95], suggesting a cell-protective function.

2. PLKs and Tumor Development

Commonly, *PLK1* and *PLK4* have been considered as oncogenes whereas tumor-suppressive functions are attributed to *PLK2*, *PLK3*, and *PLK5*. With increasing knowledge, it becomes evident, however, that the participation and the mechanisms of action of the polo-like kinase family members in carcinogenesis are much more diverse and complex and far away from being fully understood.

2.1. PLK1 Is a Dual Game Player in Tumorigenesis

The role of PLK1 in the context of tumorigenesis is complex and conflicting. On the one hand it is considered as an oncogene closely linked to human cancer development and on the other hand tumor suppressor functions are attributed to PLK1.

2.1.1. Oncogenic Potential of PLK1

According to the critical regulatory role of PLK1 during mitosis, expression of *PLK1* is below a threshold of detection in normal tissues with low proliferation rates like heart, lung, or brain. Contrary, *PLK1* levels are high in actively proliferating cells and tissues like testis, bone marrow, spleen or thymus, during embryonic development and, of special interest, in tumor cells [2,97,98]. Dysregulation of *PLK1* is a hallmark of a multitude of cancers [99,100]. Since the first report of elevated *PLK1*-levels in human cancer compared to normal, non-transformed cells [97] and malignant cell transformation initiated by constitutive expression of *PLK1* [101], *PLK1* has been shown to be overexpressed in a multitude of malignancies (reviewed by [102]) such as lung cancer [103–105], esophageal and gastric cancer [106–108], oropharyngeal carcinomas [109,110], breast cancer [103,104,111,112], melanoma [113–116], various non-melanoma skin cancers [117], (colo)-rectal cancer [118–121], medulloblastoma [122], mesothelioma [123], glioma [124], neuroblastoma [125,126], endometrial cancer (reviewed by [127]), thyroid cancer [128], pancreatic cancer [129], prostate cancer [130,131], hepatoblastomas [132], hepatocellular carcinomas [133–135], non-Hodgkin lymphomas [136,137], bladder [138] and renal cancer [139], and ovarian cancer [2,140–144]; for detail see below). High levels of *PLK1* are widely linked to oncogenic transformation, cancer progression, invasiveness, high metastatic potential and, importantly, poor overall patient survival [7,17,26,102,104–106,108–113,120,125,127,130,132,134,135,139,144,145]. *PLK1* expression has been commonly considered as a key player in cancer development capable of serving as a tumorigenic biomarker and poor-prognostic predictor (reviewed by [127]). This commonly led to the consideration of *PLK1* as a classical oncogene.

Nevertheless, the precise role of *PLK1* in the context of carcinogenesis—driving transformation by itself or solely contributing to an already initiated or established transformation—is under debate. In the light of highly proliferating tumor cells and the cell cycle-dependent expression of the mitotic kinase, high expression of *PLK1* may not be a cause but a consequence during tumorigenesis just reflecting high mitotic rate [146]. Concordant with the oncogenic conception, however, convincing evidence is accumulating that PLK1 is actively involved in the fatal events throughout tumorigenesis from the onset of oncogenic transformation, tumor growth, epithelial-mesenchymal transformation, tumor cell invasion and metastasis and, last but not least, PLK1 is supposed to participate in development of therapeutic resistance [46,146,147] and reviewed by [148]. PLK1 directly participates in oncogenic signaling and interacts with a variety of oncogenic pathways (for reviews see [102,147,149]). Important in this context is the complex and multifaceted intertwining relationship between PLK1 and several transcription factors. Among these

is the p53 protein encoded by the key tumor suppressor gene *TP53* which is a critical target of PLK1 (reviewed by [150]). Loss or mutation of the genome-guarding *TP53* and impaired p53 function account for the majority of tumor cells [151], reviewed by [152] and without functional p53, downregulation of *PLK1* is impaired leading to elevated PLK1 levels [102,111,149]. Overexpression of *PLK1* accelerates the cell cycle, enables cells to override cell cycle checkpoints leading to mitotic defects and favoring chromosomal instability and aneuploidy [48,99,100]. In a PLK1-p53 negative feedback-loop, overexpression of *PLK1* is, in turn, linked to inhibition of p53 function promoting tumorigenic status [153]. *PLK1* knockdown via RNA interference leads to *p53* upregulation in an ovarian cancer xenograft model [154]. A positive feedback-loop exists between PLK1 and the oncogenic transcription factor MYC [153]. Another tumorigenic molecular mechanism of PLK1 is promoting the inactivation of tumor suppressors like the phosphatase and tensin homologue *PTEN* [149,155]. Disruption of *PTEN* frequently occurs in cancer (reviewed by [49]). *PLK1* expression may even critically regulate *PTEN* expression acting bi-functionally [156]. Besides TP53 and PTEN, PLK1 interacts with several other tumor suppressors and oncogenes like Forkhead box protein M1 FOXM1 and members of the MYC family (for reviews see [102,147]). Another oncogenic mechanism of PLK1 is targeting metabolic pathways in order to establish a tumor-adapted and growth-favoring cellular metabolism [155,157,158]. Last not least, *PLK1* overexpression is critically involved in epithelial-mesenchymal transition, whereby well-differentiated epithelial cells acquire characteristics of rather poorly differentiated mesenchymal cells as a prerequisite for cancer cell motility, invasiveness, dissemination and metastasis. Epithelial-mesenchymal transition is one of the key processes in aggressive tumorigenesis [159,160]. This fatal transformation implies profound and dynamic alteration and re-organization of the actin-myosin and intermediate filament cytoskeleton including cell-cell and cell-matrix junctions [161,162]. *PLK1* overexpression induces and is linked to these epithelial-mesenchymal transition-associated key events via activation of defined and cancer-type specific signaling cascades [163–165]. These may include AKT, FOXM1- and MAPK-dependent pathways.

2.1.2. Tumor Suppressor Potential of PLK1

In contrast to the "pro-tumor" oncogenic potential of PLK1, there is strong evidence for PLK1 also acting as a tumor suppressor. Reduced PLK1 levels have been linked to tumorigenesis in *Plk1*-heterozygous mice [21], though in other PLK1-reduced settings a cancer-promoting effect of low and drastically reduced PLK1 could not be detected [22,98,166]. For some human malignancies such as breast cancer, elevated PLK1 levels are beneficial and associated with better prognosis [111] though reduced levels have also been reported to be exacerbating in this cancer entity [104]. With respect to breast cancer development, a tumor suppressor function of PLK1 has been confirmed in a *Plk1* gain of function mouse model using an inducible knock-in-setting [100]. For colorectal cancer, overexpression of *PLK1* is indicative of a favorable prognosis [167] with the same effect being revealed for colorectal tumors in a *Plk1*-inducible loss of function mouse model [99].

A possible explanation for these contradictory functions of PLK1 could be differences in the genetic background of cancer cells and tumor tissue. In colon cancer with chromosomal instability due to a non-sense APC mutation, elevated PLK1 levels have tumor-suppressive potential and increase the survival of patients [99]. A pan-cancer mRNA sequencing data analysis correlates *PLK1* expression levels with clinical parameter (patient overall survival) and reveals striking differentiated tumor-specific relations. Elevated PLK1 levels are associated both with good prognosis (e.g., in rectal adenocarcinoma, lung squamous cell carcinoma, and thymoma) and bad prognosis (e.g., in lung adenocarcinoma, bladder carcinoma, and kidney clear cell carcinoma) or are obviously not correlated (e.g., in stomach adenocarcinoma, cervical carcinoma, and ovarian cancer) depending on cancer type (reviewed by [149]). This diverging role of PLK1 reflects best the role of tumor heterogeneity and highly dynamic tumor change during growth and progression as well as

during treatment. The role of PLK1 in ovarian cancer is a special interest of this review and will be discussed further below.

2.2. PLK2 and Tumorigenesis

The role of PLK2 in tumorigenesis is rather complex (reviewed by [47]). *PLK2* is downregulated in several cancers and therefore has been considered as a tumor suppressor [67]. This accounts for glioblastoma [168], hepatocellular carcinoma [169], acute myeloid leukemia [170], and for B-cell malignancies [171] where *PLK2* is epigenetically silenced, as well as for cervical cancer where PLK2 exhibits anti-proliferative and apoptosis-promoting effects [172]. This accounts for gastric cancer, too, according to siRNA-mediated *PLK2* knockdown [173]. In breast cancer, reduced *PLK2* expression is linked to adverse prognosis [174]. In colorectal adenocarcinomas, *PLK2* expression is completely or partially lost [175].

PLK2 gene is targeted by p53 and it is induced after genotoxic stress in vivo [67]. In view of the frequent disruption and inhibition of the genome-guarding p53 pathway in a multitude of cancers (reviewed by [176]) tumor suppressor functions of PLK2 are impaired, too. In glioblastoma, PLK2 is negatively correlated with the central notch signaling pathway [168]. Downregulation of *PLK2* promotes tumor aggressiveness and chemo resistance via activation of notch axis and *PLK2* overexpression reduces malignant behavior both in vitro and in vivo.

The role of PLK2 in tumorigenesis is, however, conflicting. In colorectal carcinomas, PLK2 exerts tumor growth-promoting and apoptosis-inhibiting effects [167,175,177,178] as is the case for several other tumors like lung cancer, cholangiocarcinoma, osteosarcoma, and head and neck carcinoma [66,155,179–182]. An oncogenic function is also suggested for pancreatic cancer according to siRNA-induced *PLK2* silencing [183].

PLK2 has been demonstrated to mediate hedgehog survival signaling [180]. For certain cancers, as it is the case for cholangiocarcinoma, hedgehog signaling pathway is essential conferring apoptotic resistance. In colorectal cancer, PLK2 targets the Fbxw7/Cyclin E pathway promoting G1 transition to S phase of the cell cycle. High expression of *PLK2* correlates with high expression of Cyclin E promoting tumorigenesis [177]. In the same malignancy, the transcription factor forkhead box family member FOXD1 promotes *PLK2* expression on mRNA and protein levels resulting in increased proliferation and suppressed apoptosis of cancer cells [178].

Besides tumor-associated roles, PLK2 is critically involved in the pathogenesis of Alzheimer's disease as elevated levels promote production of amyloid beta plaques in a mouse model [184] and also in synaptic plasticity [59]. The role of PLK2 in ovarian cancer is of special interest for this review and will be discussed further below.

2.3. PLK3 and Tumorigenesis

PLK3 is critically involved in oncogenesis (reviewed by [72]) and dysregulated expression of *PLK3* is found in a variety of tumors.

Expression of *PLK3* is reduced in various cancers like lung [71,185,186], hepatocellular carcinoma [169], head and neck squamous cell carcinoma [187], anal squamous cell carcinoma [74] as well as melanoma, liver, kidney, stomach, rectum [81], colon tumors [188], bladder, and uterus cancer [186]. Therefore, PLK3 is widely considered as a tumor suppressive kinase [26,68]. Supportive for this conception is the apparently more frequent development of spontaneous tumors in aged *Plk3*-deficient mice [68] although the correlation between *PLK3*-deficiency and higher tumor incidence is questioned [69].

Elevated expression of *PLK3*, however, is reported to account for breast cancer [104], prostate cancer [189], and hepatoblastoma [186] and is associated with poor prognostic impact. In colorectal cancer, overexpression of *PLK3* has been shown to correlate with unfavorable prognosis based on gene expression analysis [167]. Increased *PLK3* expression corresponds with better disease-related outcome in treated cervical carcinoma and low *PLK3* expression is associated with therapeutic resistance and increased metastasis based

on immunohistochemical and genomic analyses [190] making PLK3 and its substrate pT273 Caspase 8 a valid prognostic marker.

The reasons for these differential cancer type-related and possibly development-associated expression patterns of *PLK3* remain elusive. PLK3 has been found to be a critical player in cellular hypoxic responses, which contribute to tumorigenesis and tumor progression [191–193]. The intimate involvement of PLK3 in various stress responses including DNA damage response implies cell cycle arrest and induction of apoptosis [72,79]. In this context PLK3, mediates p53-dependent and p53-independent pathways [72,77,194]. PLK3 functions in a pro-apoptotic response after induction by transcription factor NFκB [195].

Plk3-deficient mice frequently develop tumors which are intensely vascularized [68]. This points to an essential role of PLK3 as a mediator of tumor angiogenesis, which is a prerequisite for tumor growth [192]. PLK3 acts via phosphorylating hypoxia inducible factor HIF-1α as a key protein in the angiogenic pathway [81]. The role of PLK3 in ovarian cancer is of special interest for this review and will be discussed further below.

2.4. PLK4 and Tumorigenesis

As accounts for *PLK1*, *PLK4* is overexpressed in a variety of solid tumors and hematologic malignancies considered to act as an oncogene (for reviews see [11,89]). High levels of PLK4 correlate with tumor growth, aggressive progression and treatment resistance representing a poor-prognostic marker. This accounts for a variety of malignancies including several epithelial cancer types (reviewed by [11,89]) such as breast cancer [196–199], lung cancer [200,201], prostate cancer [202], and gastric cancer [203,204], but also for neuroblastoma [205], glioblastoma [206], and acute leukemia [207,208]. In skin epidermis, overexpression of *PLK4* leads to hyperplasia [209] and promotes—in association with p53 deficiency—tumorigenesis causing hyperproliferation and abnormal differentiation of basal keratinocytes and melanocytes [209,210]. Additionally, melanoma overexpress *PLK4* [211]. For colorectal cancer, data concerning effects of *PLK4* overexpression are conflicting as it has been shown to be related to a favorable prognosis [167] as well as to an adverse disease development and unfavorable prognosis [212–214]. In hepatocellular carcinoma, downregulation of *PLK4* is reported to be linked to larger tumor size and poor prognosis [40,169] as it has been observed for overexpressed *PLK4* [213]. These contradictory correlations point to PLK4 possibly acting as a tumor promoter as well as a tumor suppressor depending on cancer biology.

In view of the pivotal role of PLK4 in centriole duplication, mitotic progression, and cytokinesis, dysregulated *PLK4* promotes oncogenic mitotic errors and genomic inconstancy. $Plk4^{+/-}$ heterozygous mice are viable but frequently develop spontaneous lung and liver tumors compared with $Plk4^{+/+}$ homozygous mice [215]. Overexpressed *PLK4* induces centrosomal amplification and disturbs cytokinesis while downregulated expression of *PLK4* disturbs formation of the spindle apparatus [84,91,209,210]. Both lead to chromosomal instability, which implies a high rate of chromosome abnormalities (reviewed by [216]), aneuploidy and aberrant cell proliferation, hallmarks of human cancer cells [203,217,218]. Chromosomal instability and aneuploidy is negatively correlated with aggressiveness and metastasis of cancer, with adaptive resistance to therapies and with bad prognosis [219–221]. Amplificated centrosomes are sufficient to induce aneuploidy and to promote spontaneous tumorigenesis possibly involving downregulation of the p53 pathway [222]. Overexpressed *PLK4* has been shown to activate the ataxia telangiectasia and Rad3-related (ATR)-checkpoint kinase 1 (CHEK1) signaling pathway, which is critically involved in DNA damage response and genomic stability [213]. This pathway is supposed having a tumor-promoting function in several cancers. In addition, PLK4 induces epithelial-mesenchymal transition (for reviews see [11,89], which is one of the key processes in aggressive tumorigenesis as depicted for oncogenic function of overexpressed *PLK1* (see above). Cancer cell motility and invasiveness imply dynamic cytoskeletal re-organization and junctional cell-cell as well as cell-matrix (re)modelling [161,162]. *PLK4* overexpression affects normal intercellular adhesion and promotes invasiveness through activation of onco-

genic signaling pathways [223]. PLK4 drives epithelial-mesenchymal transition of cancer cells, cancer cell motility and migration. PLK4 acts via the phosphatidylinositol 3′ kinase PI3K/AKT pathway and via the actin-related protein ARP 2/3 complex mediating actin cytoskeletal rearrangement and aiming at cadherin-conveyed cell adhesion [205,224–226]. PLK4 is also part of WNT signaling pathways involved in the regulation of cancer cell motility [227] and invasiveness [214]. In addition, PLK4 may be responsible for development of drug resistance to taxane-based neoadjuvant chemotherapy through the induction of tubulin-mutations [199]. The role of PLK4 in ovarian cancer is of special interest for this review and will be discussed further below.

2.5. PLK5 and Tumorigenesis

PLK5 acts as a tumor suppressor in human brain cancer as it is silenced in glioblastoma [96]. A defined mutation of *PLK5* is specifically implicated in metastasis of human renal cell carcinoma [228].

3. Targeting PLKs in Cancer

In view of the multilayered significance of dysregulated PLKs in the context of tumorigenesis, family members are targeted in cancer treatment. This accounts especially for *PLK1* and *PLK4* as they are selectively overexpressed in a broad range of tumor cells and tissues compared to healthy ones (for reviews see [7,11,17,148,229]).

Since the first reports of *PLK1* downregulation with anti-sense oligonucleotides and small interfering RNA inducing growth inhibition in cancer cells [230–232] and in living animals [27,232] a plethora of studies have been performed focusing on PLK1 inhibitors suitable as anti-cancer drug [233–237] reviewed by [238–240]. Inhibiting PLK1 activity addresses actively dividing cells and leads to mitotic arrest at the G2-M transition, double-stranded DNA breaks and to induction of apoptosis [51,115,116,125,131,208,241–243]. Promising inhibitors targeting PLK1 have been investigated, approved in preclinical studies and some have reached clinical trials [240]. Remaining problems are limited therapeutic efficiency, cancer recurrence, development of resistance and side effects due to low specificity. It is important to realize that PLK1 inhibitors that are currently in the clinic might target to some extent rapidly dividing cell irrespective if they are malignant or healthy [150,242]. The third generation ATP-competitive PLK1 inhibitor, Onvansertib®, was tested in an array of 260 kinases, showed very high specificity toward PLK1 and could represent a major improvement compared to previous generations of ATP-competitive PLK1 inhibitors.

Three classes of PLK1 targeting drugs can be distinguished, namely ATP-competitors, polo-box domain competitors and RNA interference-based therapies. ATP-competitors address the kinase domain carrying the ATP-binding pocket. This domain is, however, shared by all family members (except PLK5) and by many other kinases. Therefore drugs targeting this pocket address also other kinase domains affecting, among others, the tumor suppressor functions of PLK2 and in particular of PLK3 leading to adverse side effects [244]. The most widely used (small molecule) inhibitors targeting the catalytic activity of PLK1 are BI2536 and BI6727 (Volasertib®) for reviews see [148,240]. Contrary, inhibitors targeting the unique polo-box domain of PLK1 reach higher specificity and minimize cross-reactivity with other kinases. The first designed polo-box domain-targeted inhibitor (Poloxin®) induces mitotic arrest [148,245–247] and inhibits tumor growth in vivo in a xenograft mouse model [248]. Several PLK1 polo-box domain competitors followed [148,207,240,249].

A promising and effective strategy for specific targeting of PLK1 without affecting other kinases, is the use of RNA interference (RNAi). RNAi drugs induce gene silencing by sequence-specific cleavage of targeted mRNA. The specificity as well as efficiency of this strategy has been demonstrated in a genetically engineered mouse model via a short hairpin RNA (shRNA) induced *Plk1* mRNA knockdown considerably reducing side effects [98,250]. Specific *PLK1*/PLK1 knockdown on both protein and mRNA level is achieved by short interfering Ribonucleic Neutrals (siRNNs) as pro-drugs which can be cleaved intracellularly into short interfering ribonucleic acids (siRNAs) as demonstrated

in leukemia [251]. Effective and specific siRNA-*PLK1* inhibition could also be achieved in prostate cancer cells [131] and in acute myeloid leukemia [54]. Selective knockdown of *PLK1* mRNA is induced by siRNNs in acute lymphoblastic leukemia [208].

siRNA-silencing of *PLK2* gene promoted apoptosis during mitosis in various cancer cell lines (e.g., carcinoma of lung, cervix, breast and colon, and osteosarcoma) in the presence of spindle poisons like paclitaxel [67]. *PLK2*-silencing does not impair mitotic cycle progression in cells not exposed to microtubule poisons. Therefore, inhibition of PLK2 might be a therapeutic option for sensitizing Paclitaxel®-resistant tumors [67]. In cholangiocarcinoma, pharmacological inhibition of PLK2 via BI6727 and *PLK2* knockdown degrade the anti-apoptotic protein myeloid cell leukemia 1 (Mcl-1) which represents a survival factor in this malignancy, and lead to apoptosis and tumor suppression in vivo [180].

In view of the critical role of *PLK4* overexpression in a variety of malignancies, its inhibition is a therapeutic cancer strategy [10,11,91,229] for reviews see [11,89]. Several small molecules ATP competitive inhibitors have been developed, among which CFI-400945 is the most prominent. It affects centriole duplication and mitotic spindle formation, prevents cellular abscission and generates polyploid cells resulting in apoptotic death as demonstrated in breast cancer and colorectal cancer lines [91]. CFI-400945 inhibits lung cancer growth in mice [200]. Preclinical studies confirmed in vivo anti-tumor activity as it is the case for patient-derived xenograft (PDX) models, breast cancer, pancreatic cancer, and osteosarcoma [252,253]. Favorable results are obtained from a clinical study with various solid tumors [254]. Other inhibitors target the critical role of PLK4 in centriolar function and inhibit cell proliferation in melanoma cells [211], breast cancer [255], cervical carcinoma, and colon carcinoma [256,257] only to name a few.

4. Ovarian Cancer and PLKs

As normal ovarian tissue contains actively proliferating cell populations, *PLK1* is physiologically expressed at high levels [2,140–143]. The other members of the PLK family, especially *PLK2* and *PLK3*, are differentially expressed in periovulatory granulosa cells via hormonal induction [258].

Treatment of ovarian cancer representing the most lethal gynecological malignancy worldwide is a tremendous challenge [259]. Until now an effective screening strategy is missing to detect ovarian cancer at an early developmental stage, so it is often diagnosed at an advanced stage with bad prognosis due to aggressive metastatic progression, relapse after surgery and development of therapeutic resistance. Ovarian cancer comprises several divergent subtypes according to histological characteristics, molecular features, origin of cells (ovarian or extra-ovarian), risk factors, clinical features, and therapeutic response (for reviews see [260–263]). The most common subtype are epithelial ovarian carcinomas (about 90%) including the minor frequent subtypes mucinous, endometroid, clear cell subtype and the so-called serous ovarian carcinoma (about 75%) with a low-grade and high-grade variant of all serous ovarian carcinomas (about 70%). These various subtypes of ovarian cancer bear a multitude of significant molecular aberrations and genomic changes and are genetically marked heterogeneous as revealed by integrated genomic analysis based on the Cancer Genome Atlas [264] and other studies [262,265–267]. The specific genotype critically determines the response to therapeutic treatment [268,269].

4.1. PLK1 in Ovarian Cancer

As accounts for a multitude of other cancers, dysregulation of *PLK1* is linked to ovarian cancer. High expression of *PLK1* is reported to be associated with histological grade and clinical stage [143]. Elevated levels of *PLK1*/PLK1 mRNA and protein account for ovarian cancer cell lines and tissue and promote growth and migration of cancer cells and diminish apoptosis [270]. Overexpression of *PLK1* correlates positively with mitotic activity and is a prognostic factor in ovarian carcinoma linked to worse patient prognosis as judged from immunohistochemistry of several malignant epithelial subtypes [130]. This correlation is confirmed in a *PLK1* knockdown xenograft model pointing to PLK1 as an independent

prognostic factor [271]. Highly expressed *PLK1* promotes proliferation and migration of cultured ovarian cancer cells [272]. In a large patient cohort with early-stage ovarian cancer, high *PLK1* expression correlates with bad prognosis based on immunohistochemistry [273]. Importantly, pharmacological induction of strong mitotic arrest via PLK1 inhibition and microtubule targeting followed by blocking mitotic exit activates apoptosis, prevents endoduplication and reduces chromosomal instability in an ovarian cancer cell line culture system [273]. Indirect evidence for the tumor-promoting effect of overexpressed *PLK1* in (relapsed) ovarian cancer comes from the induction of synthetic lethality in a patient-derived ovarian cancer cell culture [274] and from the clinically approved antitumor effect of the PLK1 inhibitor B16727 (Volasertib®) [275]. In a pan-cancer analysis using mRNA quantitative sequencing data, however, differing correlations between *PLK1* upregulation and overall survival of patients suffering from different cancer entities have been shown with *PLK1* overexpression not influencing overall survival for ovarian cancer (reviewed by [149]). In the mucinous subtype of ovarian carcinoma, downregulation of *PLK1* with siRNA as well as pharmacological PKL1 inhibition (Volasertib® and Onvansertib®, both highly selective ATP-competitive PLK1 inhibitors) [276] in a xenograft model interferes with cell proliferation inducing mitotic arrest at G2/M phase leading to endoduplication as well as apoptosis [277]. The complex diversity of PLK1′s role in ovarian cancer becomes obvious in light of PLK1 serving as predictive marker for better prognosis with regard to the progression-free survival in a stage-dependent manner: PLK1 functions as a positive predictor in early-stage of a low-grade serous subtype but not in late-stage based on mRNA and protein expression [144]. Transcriptomic analysis of serous ovarian carcinomas reveals PLK-signaling events and PLK-dependent differentially expressed genes to be important in tumorigenesis and cancer progression [278].

Aurora borealis (*BORA*) is highly expressed in aggressive ovarian cancer and exerts its oncogenic role via activation of *PLK1* in vitro and in vivo [279]. BORA activates the key mitotic PLK1 function as a prerequisite for mitotic entry and G2/M checkpoint recovery and high BORA correlates with increased cancer cell proliferation and high grade of chromosomal instability [279]. One example of the complex network integration of PLKs is the interplay with microRNA (miRNA). miRNAs are short-sequence and not protein-encoding RNAs specifically binding to target mRNAs leading to their degradation or to inhibition of translation, thus post-transcriptionally regulating gene expression. miRNAs play important anti-oncogenic as well as oncogenic roles in the pathogenesis of ovarian cancer (for reviews see [280,281]). MiR-545 directly targets *PLK1* mRNA and inhibits *PLK1* expression in ovarian cancer thereby acting as a tumor suppressor as demonstrated in vitro and in vivo in a xenograft mouse model [270]. MiR-545 functions as a tumor suppressor and is lowly expressed in epithelial ovarian tissue whereas overexpression leads to inhibition of growth and increase in apoptosis [282].

4.2. PLK2 in Ovarian Cancer

PLK2 downregulation is linked to ovarian tumorigenesis and drug resistance. In a *PLK2* knock-in and -knockdown model using primary cell culture, it could be demonstrated that therapeutic drug resistance is associated with transcriptional silencing of the kinase [283]. This was confirmed in a follow-up study [284] and is in accordance with significant downregulation of the *Plk2* gene identified in chemo resistant ovarian cancer via oligonucleotid microarrays [285]. Critical involvement of upregulated *PLK2* in resistance development is underlined by a transcriptome monitoring in isogenic ovarian cancer cells with gradually changing resistance [286].

4.3. PLK3 in Ovarian Cancer

Contrary to many other cancers (see above), *PLK3* seems to act as an oncogene in the ovary. In different types of ovarian carcinoma, *PLK3* has been found to be overexpressed and to correlate with mitotic activity without being used, however, as a prognostic factor [130].

4.4. PLK4 in Ovarian Cancer

PLK4 seems to promote ovarian cancer. High expression of *PLK4*/PLK1 on mRNA and protein level is linked to an advanced pathological stage in epithelial ovarian cancer and *PLK4*-transfected ovarian cell lines show accelerated proliferation [287]. PLK4 is considered to serve as a biomarker and poor prognostic predictor in advanced stage of disease as well as therapeutic target.

4.5. PLK 5 in Ovarian Cancer

Allele-loss on *PLK5*-carrying chromosome locus is associated with a 50% increased risk of sporadic ovarian tumors among other neoplasia [288], suggesting a tumor-suppressive function of PLK5 in the ovary.

5. Targeting PLKs in Ovarian Cancer

Targeting PLKs in ovarian cancer bears diagnostic and prognostic potential as well as powerful therapeutic options. Late diagnosis of ovarian cancer at an advanced stage of disease is one cause of the high mortality rate. An important strategical goal must be the identification of markers suitable to detect ovarian cancer at early stage. PLK-dependent differentially expressed genes may serve as attractive biomarkers with prognostic value for early detection [278].

In order to effectively address tumor-specificity, a better staging of ovarian cancer including molecular markers for patient stratification is needed [289]. This is necessary in order to choose the appropriate treatment and to optimize that is personalize, therapeutic regime. Besides Ki67, PLK1 is a suitable stage-dependent marker. At least in a subtype of serous ovarian cancer this could be demonstrated on the level of mRNA expression [144]. The introduction of DNA microarray technique for determination of PLK levels in clinical use might be of strategical benefit.

The standard therapy for advanced disease (as most women present) is based on debulking surgery and chemotherapy with platinum derivatives (cisplatin, carboplatin) and taxanes, esp. Paclitaxel® [290]. The former bind to DNA and inhibit DNA replication and transcription thus terminating cancer cell growth, the latter hyperstabilize microtubules perturbing mitotic spindle formation and correct chromosome segregation, which activates the mitotic spindle assembly checkpoint and prolongs mitotic arrest or inhibits mitosis. After initial responsiveness to chemotherapy most patients suffer from cancer recurrence with enhanced metastatic aggressiveness, acquired drug resistance, poor prognosis and ultimate death [291]. The reasons for this fatal course are complex and under intense examination. An important key to understanding is the marked inter- and intra-tumor heterogeneity of ovarian cancer bearing a multiplicity of mutations [264] and transcriptional silencing of many genes [292] affecting numerous key signaling pathways [293–296]. A very high degree of genome instability is characteristic, too [297]. Ideally, chemotherapy leads to death of these cancer cells. Affected cells, however, may not die via apoptosis but escape via mitotic slipping and continue cycling [298,299]. This means survival of cancer cells with an abnormal aneuploid genome and a high grade of chromosomal instability [300] driving fatal relapse with pronounced aggressiveness and metastasis. In particular, surviving ovarian cancer stem cells are thought to harbor malignancy and drug resistance [301]. In view of noteworthy populations of cancer cells not being killed by standard chemotherapy it is an urgent necessity as well as a tremendous challenge to optimize specified targeting. One strategy is addressing ovarian cancer-specific signaling pathways [293,294,301]. This essential knowledge may be beneficial in adjusting and personalizing therapy. A very promising strategy is a drug treatment regime including PLK1-inhibition. A therapeutic option is, among others, Volasertib® (BI 6727), an ATP analogue designed as a PLK1-inhibitor targeting and inhibiting the kinase domain. Anticancer activity of Volasertib® has been shown in patients with platinum-resistant or -refractory ovarian cancer [275]. Volasertib® in a dual combination with the standard microtubule-targeting taxane, paclitaxel, induces synthetic lethality in different ovarian cancer cell lines including patient-derived ones [274]. This

approach led to a significant increase in polyploid cells [273]. Pharmacological inhibition of PLK1 by BI2536 or Volasertib® has been shown to induce mitotic arrest and slippage in a dose-dependent manner, that is with high concentration of PLK1 inhibitor [242]. BI 2536 was the first selective PLK1 inhibitor used in clinical trials for the treatment of advanced ovarian cancer [302]. Finally, Volasertib® in combination with paclitaxel induces strong mitotic arrest in ovarian cancer cell lines and, when followed by pharmacological blocking of mitotic exit, prevents endoreduplication and reduces chromosomal instability [273]. This effective triple-combined strategy underlines the potency of PLK1 inhibition in the treatment of ovarian cancer. In mucinous ovarian carcinoma, specific downregulation of *PLK1* via siRNA and pharmacological inhibition via Volasertib® or Onvansertib® inhibits growth of cultured cell lines and induces apoptosis [277]. In transfer to an in vivo xenograft model best tumor growth inhibition is achieved via Onvansertib® synergistically combined with paclitaxel.

Silencing of *PLK2* dramatically sensitizes an ovarian cancer cell line to anti-microtubule agents leading to apoptosis during mitosis [67].

A kinome-wide screening for modulators of the growth-inhibitory effect of cisplatin revealed that the inhibition of PLK3 sensitizes cultured ovarian cancer cells to chemotherapy [303].

In several ovarian cancer cell lines, inhibition of PLK4 kinase activity with the small-molecule inhibitor CFI-400945 inhibited proliferative activity and induced polyploidy [91]. Targeting PLK4 with the small molecule inhibitor YLZ-F5 prevents human ovarian cancer growth by inducing aneuploidy and promotes apoptosis via activation of caspases-3/9 and impairs cell migration [304].

6. Mouse Models in Ovarian Cancer Research

Important tools for the progress in ovarian cancer research are well-suited models. Besides classical methods using cell culture, mouse models based on xenografts and on genetic engineering are indispensable in ovarian cancer research [262,305]. They address the origin of ovarian cancer, genetic profiles and mutations, tumor growth and metastasis, effects of putative treatments as well as (therapeutic) response.

Xenograft mouse models can be designed with patient-derived grafts or with well-defined cancer cell line grafts [306,307]. They are commonly used to gain knowledge about ovarian tumor biology and to screen therapeutic drug efficiency. Their value depends, besides above-mentioned types of graft, on the immune status of the host (immunodeficient or not) and on the method of engraftment. A common procedure is heterotopic engraftment, most commonly subcutaneous inoculation of ovarian cancer cells or tissue enabling rapid tumor growth easy in observation and measurement [277] (Figure 3).

However, subcutaneous tumor growth does not represent physiological environment. Interactions between non-malignant stromal cells surrounding and infiltrating tumors have been shown to critically determine tumor growth, spreading and response to therapy and are therefore decisive with respect to patient prognosis [154,308,309]. To get closer to a clinically relevant pathophysiological macro- and microenvironment, intraperitoneal tumor cell transplantation or an orthotopic model is preferable [310]. Tumor cells or a solid tumor are applicated intrabursally, i.e., into the bursa ovarica enclosing mouse ovary or directly onto the ovary, thereby reproducing the primary site of tumor growth and disease progression best [311]. Intrabursal as well as intraperitoneal inoculation allow tumor cells to spread throughout the peritoneal cavity and to attach to serosal surfaces thus representing a well-suited metastatic model [312,313]. It is, however, worth to consider that the bursal enclosure of mouse ovary does not apply to human ovary which restricts accurate imitation of metastasis. In vivo monitoring of tumor growth and spreading is repeatedly possible using a bioluminescent marker and detection system. Transferred tumor cells or tumor pieces are stably transfected with luciferase, thereby generating a luminescent signal upon injection of luciferin as a substrate. The signal can be visualized using a bioluminescent imaging technology [314–316]. This technique allows in vivo tracking of cells over a long time. Additionally, magnet resonance imaging (MRI) can be

used to track cancer cells in mouse models [317,318]. Xenograft models, however, often have the disadvantage of an immune-deficient setting not being able to consider (micro)-environmental tumor interactions. Syngeneic models using genetic engineered murine cell lines for engraftment [319] partially overcome this restriction as applies for humanized mouse models containing human immune cells [307].

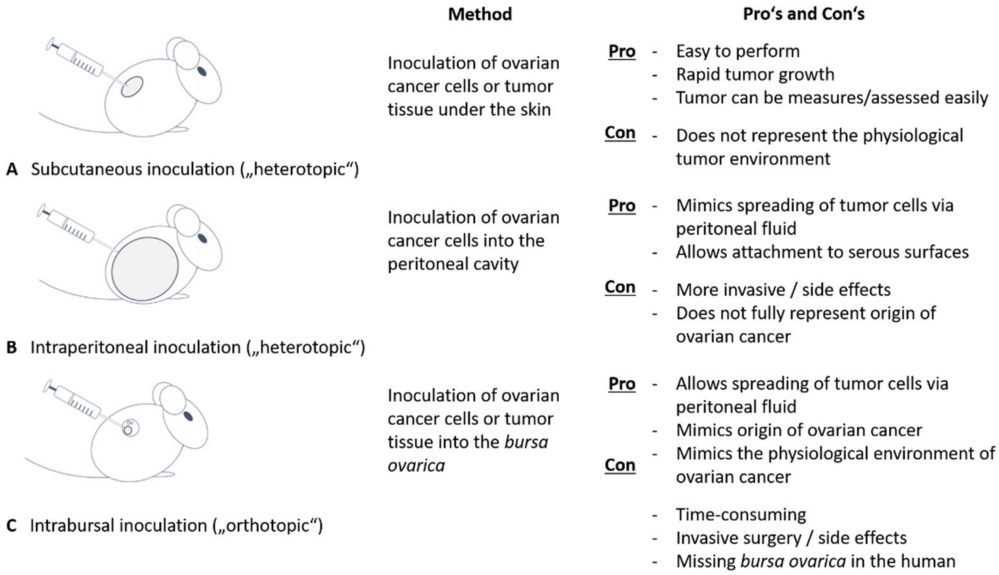

Figure 3. Mouse models in ovarian cancer research. Using mouse models have proved to be an important tool in ovarian cancer research. We differentiate between heterotopic mouse models, where ovarian cancer cells or cancer tissue is inoculated subcutaneously (**A**) or into the peritoneal cavity (**B**). Whereas a subcutaneous application is easy to perform and safe for the animal, it does not mimic the physiological tumor environment. In contrast, intraperitoneal application is able to imitate the spreading of tumor cells via peritoneal fluid and attachment to serous surfaces. As an orthotopic mouse model, intrabursal application is performed (**C**). The latter also imitates site of origin of ovarian cancer and mimics spreading and attaching of cancer cells, but is hampered by the fact that an ovarian pouch is missing in the woman.

Genetically engineered mouse models (GEMMs) for ovarian cancer offer the great chance to precisely predetermine genetic modifications [320,321]. In view of the complex histological and molecular diversity of ovarian cancer it is challenging to establish subtype-mimicking GEMMs that recapitulate geno- and phenotypic features of human cancer. Differentiated models have been developed considering the side of cancer origin (fallopian tube vs. ovarian surface epithelium) as well as tumor genetics targeting critically involved genes (like *Trp53*, *Rb1*, *Pten* or *Dicer1*) and signaling pathways (like PI3K, Myk, PTEN or Wnt/β-catenin) [322–327].

Several defined syngeneic models recapitulate specific genetic and epigenetic profiles of various human ovarian cancer subtypes and offer the great opportunity to mimic cancer growth and development as well as drug response [328–330].

With respect to the exploration of the role of PLKs in cellular biology genetic mouse models using inducible knock-out or knock-in strategies have been valuable as depicted above. A powerful transgenic tool is the inducible RNA interference (RNAi) technology. The *PLK1* gene can be reversibly silenced, as demonstrated in an ovarian cancer cell line, among others [98].

7. Conclusions

Taken together, members of the multifunctional and multifaceted PLK family are critically dysregulated in a multitude of malignancies and represent powerful targets in diagnosis and effective therapy. Successful treatment of ovarian cancer remains to be a

challenge and especially PLK1 and PLK4 proof to be a very promising target for diagnosis and treatment. Well-suited mouse models are necessary to increase knowledge about tumor biology and to develop powerful therapeutic regimes. Targeting PLKs has the potential for a breakthrough technological advance and clinical success.

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