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REVIEW

Anillin is an emerging regulator of tumorigenesis, acting as a cortical cytoskeletal scaffold and a nuclear modulator of cancer cell differentiation

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Abstract

Remodeling of the intracellular cytoskeleton plays a key role in accelerating tumor growth and metastasis. Targeting different cytoskeletal elements is important for existing and future anticancer therapies. Anillin is a unique scaffolding protein that interacts with major cytoskeletal structures, e.g., actin filaments, microtubules and septin polymers. A well-studied function of this scaffolding protein is the regulation of cytokinesis at the completion of cell division. Emerging evidence suggest that anillin has other important activities in non-dividing cells, including control of intercellular adhesions and cell motility. Anillin is markedly overexpressed in different solid cancers and its high expression is commonly associated with poor prognosis of patient survival. This review article summarizes rapidly accumulating evidence that implicates anillin in the regulation of tumor growth and metastasis. We focus on molecular and cellular mechanisms of anillin-dependent tumorigenesis that include both canonical control of cytokinesis and novel poorly understood functions as a nuclear regulator of the transcriptional reprogramming and phenotypic plasticity of cancer cells.

Keywords Actin cytoskeleton · Cell adhesion · Cell migration · Cytokinesis · Tumor metastasis · Stem cells

Introduction

Oncogenic transformation results in dramatic alterations of the cellular homeostasis enabling rapid cancer growth and dissemination. Remodeling of the intracellular cytoskeleton is one of the key mechanisms involved in tumorigenesis, since the cytoskeleton directly controls two major features of cancer cells: proliferation and motility [1–4]. The cytoskeleton represents a well-organized meshwork of filaments and fibrils, created by self-assembly of different structural proteins. There are four major cytoskeletal elements in human cells: actin filaments, microtubules, intermediate filaments and septin polymers [5, 6]. These structures act together in complex physical interactions and through signaling cross-talks to control cellular architecture and functions. All major cytoskeletal elements are essential for tumor development [1, 3, 4, 7], however, the way in which the orchestrated remodeling of different cytoskeletal structures drives tumor growth and metastasis remains poorly understood. Importantly, current anticancer drugs that interfere with the cytoskeleton narrowly target microtubules, e.g. taxanes. These drugs cause severe side effects, and often tumors become resistant to the microtubule-targeting agents [8, 9]. Currently, there are no therapeutic approaches that interfere with actin or septin cytoskeleton in cancer cells. However, both actin filaments and septin polymers have crucial functions in normal cells and are likely to be poor targets for anticancer drug development. A more feasible approach would be to identify and inhibit common upstream regulators of major cytoskeletal elements that are overexpressed or hyperactivated in cancer cells. This review article focuses on a unique cytoskeletal scaffolding protein, anillin. Anillin is known to interact with actin filaments, microtubules, septins and membrane phospholipids. It is a well-recognized and evolutionally conserved regulator of cytokinesis and is commonly overexpressed in many types of solid tumors. The canonical functions of anillin during cell division and its

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emerging functions in non-dividing cancer cells are reviewed in this article. Existing evidence about anillin overexpression and functions in different cancers and its possible value as a predictive biomarker are also summarized. Finally, the idea that the anillin-dependent regulation of tumorigenesis involves its dual action as a cytoplasmic cytoskeletal scaffold and a nuclear regulator of the transcriptional program of cancer cells will be discussed.

Domain organization and binding partners of anillin

Anillin was originally isolated from *Drosophila* embryo extracts using filamentous (F) actin affinity chromatography, showing a specific binding affinity to F-actin, but not monomeric actin [10]. Subsequent studies identified anillin and anillin-like proteins in many organisms, from yeasts to humans, which display a high degree of structural and functional conservation during evolution [11]. Humans have a single anillin gene located on the Chromosome 7 and encoding a 1124 amino acid protein with estimated molecular mass of 124 kDa. Interestingly, this protein migrates as a broad band of higher size, near 180 kDa, during the SDS-PAGE electrophoresis [12–15], which may reflect yet to be defined posttranslational modifications. A shorter splice isoform of human anillin missing an exon (508–544 amino acid region), has been also reported [16], however, expressional and/or functional differences between the long and the short anillin isoforms have not been investigated. The amino-acid sequence of anillin has been well mapped identifying several domains/binding motifs essential for interactions with different binding partners [11, 13]. The actin-binding domain is located at the N-terminal part of the anillin molecule (amino acids 251–454 in human protein) and contains several actin-binding sites (Fig. 1) [17]. *Drosophila* anillin has three actin-binding sites [18] and human anillin can also bind two or more actin molecules [19]. The ability of anillin to simultaneously bind more than one actin monomer explains its preferential interactions with actin filaments and a filament-bundling activity. In addition to the actin-binding domain, the N-terminal region of anillin contains binding sites for other important cytoskeletal regulators, including a non-muscle myosin II (NM II) motor, an actin filament nucleator, diaphanous-related formin 3 (DIAPH3), and actin filament capping protein, CD2-associated protein (CD2AP) (Fig. 1) [13]. Interestingly, *Xenopus* anillin directly binds to NM II and this binding is accelerated by NM II activation (phosphorylation) but does not require interactions with F-actin [20]. Furthermore, in dividing mammalian cells, anillin regulates NM II localization at the equatorial cortex [16, 20, 21]. The described findings highlight anillin as an essential regulator of the assembly of contractile actomyosin structures at specific stages of the cell cycle. The regulation of actin filament growth could be another mechanism that drives anillin-dependent formation of actomyosin bundles. This mechanism is based on the reported anillin interactions with essential regulators of actin polymerization, such as DIAPH3 [22, 23] and CD2AP [24, 25]. While anillin binding was shown to stimulate the actin-nucleating activity of DIAPH3 [22, 23], its effects on CD2AP-dependent regulation of actin filament dynamics remain poorly understood. Interestingly, mutations of both anillin and CD2AP are associated with the development a severe kidney disease, focal segmental glomerulosclerosis (FSGS) [26, 27], which suggests that these two binding partners act together within the same functional pathway.

The C-terminal part of anillin contains a unique anillin homology domain (AHD, amino acid residues 645–980) and a pleckstrin homology (PH) domain (amino acid residues 981–1124) [13, 28]. The AHD is essential for anillin-dependent regulation of the Rho family of small GTPases.

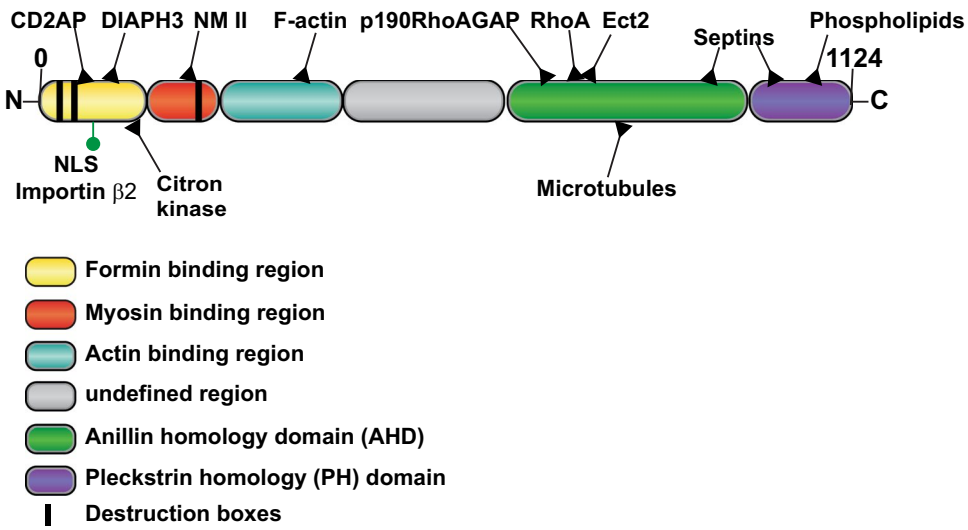

Fig. 1 Domain structure and binding partners of anillin. The structural domains and regions of human anillin molecule, along with anillin-binding partners known to interact with these domains are depicted. Other important elements of the anillin sequence, such as a nuclear localization signal (NLS) and destruction boxes are also highlighted (Adapted from [11, 13])

Indeed, a binding site for RhoA is located in the AHD (Fig. 1) [16, 28]. The same domain mediates anillin interactions with important Rho regulators, a guanine nucleotide exchange factor, Ect2 [29] and a GTPase-activating protein, p190RhoGAP [30] that activate and inhibit RhoA, respectively. RhoA's association with its upstream regulators through their interactions with anillin is thought to ensure a tight spatial control of Rho activity. Furthermore, anillin binding promotes recruitment of active Rho to actomyosin filaments to accelerate their assembly and contractility [22]. Interestingly, a microtubule-binding site either overlaps with, or localizes in the immediate proximity to, the Rho-binding site on the anillin molecule, resulting in competition between RhoA and microtubules for anillin binding [31].

The PH domain together with the adjacent part of the AHD domain have been mapped as a minimal structure that mediates anillin interactions with septin filaments [17, 32]. Since anillin can simultaneously bind F-actin and septins using different binding sites, it could serve as adaptor protein regulating side-by-side alignment of actin bundles and septin oligomers [32]. On the other hand, septin oligomers that readily bind phospholipids can mediate anillin recruitment to the plasma membrane. It is noteworthy that anillin can also interact with cell membranes in septin-independent fashion, via direct binding of negatively charged membrane phospholipids such as PIP2, mediated by its PH domain and cryptic lipid-binding sites in the AHD (Fig. 1) [28, 33]. In addition to the outlined interacting partners, anillin was shown to bind other important signaling and scaffolding molecules, some of which are included in Fig. 1. For example, human and *Drosophila* anillin physically interact with an essential regulator of cell division, citron kinase [34, 35]. Likewise, anillin binds to a cytoskeletal scaffold, IQGAP3 [36], a negative regulator of Rac GTPase, RacGAP50C [37, 38], and a membrane-curving protein, syndapin [39].

The described domain structure and multiple binding partners enable anillin to act as a versatile scaffolding protein regulating the assembly of different cytoskeletal structures at different cellular destinations. Importantly, anillin has a dynamic intracellular localization, and it can shuttle between the nucleus and cytoplasm/membrane cortex at different stages of the cell cycle [17, 40]. The nuclear accumulation of anillin is regulated by a nuclear localization signaling (NLS) motif located at the N-terminal region of its molecule (Fig. 1) [41]. This NLS motif serves as a docking site for importin β2 that transports anillin into the nucleus. While many published studies are focused on characterizing anillin-based multiprotein complexes at the cell cortex or in the cytoplasm, nothing is known about anillin interactions in the nucleus.

Anillin roles in cytokinesis

The most known evolutionary conserved function of anillin involves regulation of cytokinesis, which is a physical scission of two daughter cells at the end of mitosis. Since such anillin activity has been a subject of several excellent reviews [11, 13, 42–44], we will briefly summarize the most important findings and mechanisms. The essential roles of anillin in cell division are reflected by dramatic changes in this protein localization during the cell cycle. In cultured human, *Drosophila* cells and yeast cells, it accumulates in the nucleus during interphase but rapidly translocates to the cell cortex in metaphase, at the onset of cell division. In anaphase and telophase, anillin selectively accumulates first at the equatorial cortex and then in the contractile rings that drive cleavage furrow formation. Anillin remains associated with the ingressing furrow till the end of cytokinesis and thereafter relocalizes into the nucleus. Early studies suggested that anillin plays causal roles in cytokinesis, since its inhibition resulted in cytokinesis failure and cell multinucleation [17, 40, 45]. However, the exact roles and mechanisms of anillin-dependent regulation of cell division appear to be different in different organisms and remain incompletely understood [13, 42, 44]. In dividing vertebrate and *Drosophila* cells, anillin inhibition does not prevent the assembly of the cytokinetic furrow, but leads to its instability, lateral oscillation and eventual failure [17, 20, 21, 46]. A structural analysis and in vitro reconstitution studies of human anillin and its yeast orthologue suggest that anillin controls positioning and ingression of the cytokinetic furrow by physically linking the contractile ring to the plasma membrane [28]. According to this model, the N-terminal domains of anillin interact with actomyosin bundles of the contractile ring, whereas its C-terminal part tethers the ring to the plasma membrane via direct binding to negatively changed membrane phospholipids [28]. In addition to this scaffolding function, anillin could drive local assembly of actin filaments in the cleavage furrow by enhancing RhoA-dependent actin nucleating activity of DIAPH3 [22]. Anillin function appears to be especially important for the late stages of cytokinesis when the cortical contractile ring is transformed into the midbody ring leading to abscission. Anillin accumulates to the midbody [17, 47] and either depletion or overexpression of this protein inhibits abscission [20, 21, 35]. Mechanisms of anillin-dependent regulation of late cytokinesis are not completely understood; they could involve modulation of actomyosin contractility, since loss of anillin mislocalizes NM II from the contractile ring at late stages of the furrow ingression [20, 21].

Anillin functions in non-dividing cells

While vast majority of published papers examines the roles of anillin in regulating cytokinesis, recent studies revealed unanticipated functions of this scaffolding protein in non-dividing cells. One such function involves the regulation of epithelial barriers. Anillin is essential for the assembly of adherens and tight junctions and establishment of the paracellular barrier in human epithelial cells [14, 48] and *Xenopus* embryo [49, 50]. Such barrier-promoting effects of anillin are linked to its ability to organize the perijunctional and apical actomyosin cytoskeleton and modulate the tensile forces in this actomyosin network [14, 49, 50]. However, the mechanisms underlying anillin-dependent regulation of the perijunctional cytoskeleton remain poorly elucidated. Some evidence suggests that this protein acts as a local scaffold regulating RhoA activity and PIP2 clustering at epithelial junctions [48–50]. Alternatively, anillin could remotely control the integrity of epithelial junctions and the cortical cytoskeleton from the nucleus, by regulating MAP kinase signaling [14].

A related function of anillin involves regulation of the glomerular barrier integrity in the kidneys by modulating podocyte cell adhesion and motility [26, 51]. This function was highlighted by discovering a point mutation of the human anillin gene associated with FSGS [26, 51]. We will discuss the molecular mechanisms underlying anillin-mediated regulation of cell motility in subsequent sections focusing on tumor metastasis. Interestingly, anillin expression is high in the human adult nervous system, where the frequency of cell division is low [12]. However, very few studies have addressed anillin functions in neural cells. Thus, in *C. elegans*, anillin is essential for neuritogenesis by regulating cell migration and growth cone extension [52]. In mouse brain, anillin–septin complexes control axon myelination and accelerate nerve conduction [53, 54]. These examples highlight versatile functions of anillin in different tissues that go beyond the canonical regulation of cytokinesis.

Anillin upregulation in cancer

For more than a decade after its discovery, anillin was studied primarily by cell and developmental biologists interested in the mechanisms of cell division, without being associated with any human disease. However, in 2003, a published microarray analysis of human breast cancer tissues identified anillin as one of a limited number of genes upregulated during transition from ductal carcinoma in situ to invasive ductal carcinoma [55]. Subsequent clinical studies demonstrated anillin overexpression in many types of human solid tumors, including breast, pancreatic, colorectal, lung, gastric, urothelial and liver cancer (Table 1) [12, 56–69].

The relationships between the anillin expression and clinical–pathological parameters of the disease are well established in breast cancer subtypes. High anillin expression is associated with more aggressive estrogen and progesterone receptor-deficient (ER−, PR−) breast tumors, but appears to be independent of the human epidermal growth factor receptor 2 (HER2) status [61, 67, 70]. Furthermore, meta-analysis of gene and protein expression data demonstrates that high anillin expression is a prognostic marker of poor survival in breast cancer patients [61, 67, 71]. Interestingly, a recent study identified a 'beneficial' single-nucleotide polymorphism of the anillin gene with apparent dominant-negative functional effects [76]. Carriers of this anillin variant have lower risk of developing breast cancer, even while possessing a common tumor-promoting mutation of the BRCA1 oncogene [76]. Beside breast cancer, high levels of anillin mRNA and protein are associated with poor prognosis of patients with lung [64, 72], pancreatic [57, 65], liver [58, 69], bladder [68] and colorectal [66] cancers (Table 1). Importantly, the prognostic significance of anillin appears to be dependent on the intracellular localization of this protein. Nuclear staining of anillin negatively correlates with diseases-free survival of patients with urothelial carcinoma; however, no correlation is observed for its cytoplasmic staining [59]. Furthermore, increased cytoplasmic staining of anillin positively correlates with patient survival in renal cell carcinoma [73]. This highlights the importance of determining the intracellular localization of anillin in tissue samples obtained from different cancer patients. Such localization may not only help to establish the predictive values of anillin expression, but also to understand molecular mechanisms mediating the tumorigenic activity of this protein. In the majority of published clinical studies, anillin is predominantly localized in the nuclei of cancer cells and only a weak cytoplasmic staining is seen via immunohistochemistry (IHC) (Table 1). Likewise, immunohistochemical labeling of anillin in different tumors presented in the Human Protein Atlas shows primarily nuclear localization of this protein. Importantly, the specificity of nuclear localization of anillin in cultured cancer cells is verified by either siRNA-mediated knockdown or CRISPR/Cas9-mediated knockout of this protein [14, 15, 77]. Overall, the described clinical studies demonstrate a substantial upregulation of anillin expression in different solid tumors where it primarily localizes in the nucleus. This high nuclear expression of anillin serves as a significant prognostic factor for poor survival of cancer patients.

Anillin as a tumor growth promoting factor in vitro and in vivo

Consistent upregulation of anillin expression described in clinical cancer studies stimulated a growing interest in

Table 1 Upregulation of anillin expression in different cancers and its prognostic value for patient survival

Cancer type	mRNA or protein analysis	Anillin localization	Prognostic value	References
Breast cancer	mRNA overexpression	N/A	ND	[12]
Breast cancer	mRNA overexpression	N/A	Associated with poor survival	[70]
Breast cancer	Protein overexpression (IHC)	Nuclear	Associated with poor survival	[61, 67, 71]
Lung cancer	mRNA overexpression	N/A	Associated with poor survival	[60]
Lung cancer	Protein overexpression (IHC)	Nuclear	Associated with poor survival	[64]
Lung cancer	mRNA and protein overexpression (IHC and Western blot)	Nuclear	Associated with poor survival	[72]
Pancreatic cancer	mRNA and protein overexpression (IHC)	Nuclear and cytoplasmic	Associated with poor survival	[57, 65]
Colorectal cancer	Protein overexpression (IHC)	Nuclear	ND	[56]
Colorectal cancer	mRNA and protein overexpression (IHC and Western blot)	Nuclear	Associated with poor survival	[66]
Liver cancer	mRNA and protein overexpression (IHC and Western blot)	Nuclear and cytoplasmic	Associated with poor survival	[58]
Liver cancer	mRNA and protein overexpression (IHC)	Nuclear	Associated with poor survival	[69]
Bladder cancer	mRNA and protein overexpression (IHC)	Nuclear and cytoplasmic	Associated with poor survival	[68]
Urothelial cancer	Protein overexpression (IHC)	Nuclear and cytoplasmic	Associated with poor survival (nuclear staining)	[59]
Renal cancer	Protein overexpression (IHC)	Nuclear and peripheral	Associated with better survival	[73]
Nasopharyngeal cancer	mRNA and protein overexpression (IHC)	Nuclear and cytoplasmic	ND	[74]
Ovarian cancer	mRNA overexpression	N/A	ND	[12]
Hormone resistant prostate cancer	mRNA overexpression	N/A	ND	[75]

N/A not applicable, *ND* not determined

investigating the functional roles of anillin in tumorigenesis. Several recent publications addressed this question by utilizing common human cancer cell lines with high anillin expression and either transiently or stably decreasing anillin level in vitro using RNA interference and CRISPR/Cas9-mediated gene knockout [1, 15, 58, 61, 64, 65, 68, 69, 78]. These in vitro studies that lowered anillin expression in cancer cells examined two major oncogenic features, *e.g.,* cell growth/proliferation and cell motility. Many of the findings appear to be consistent. For example, either siRNA/shRNA knockdown or CRISPR/Cas9 knockout of anillin inhibited soft agar growth of breast [15, 78], pancreatic [65], prostate [75] cancer cells and hepatocellular carcinoma [58]. On the other hand, upregulation of anillin expression in poorly tumorigenic MCF10ANeoT breast cancer cells markedly accelerated their colony-forming ability on soft agar [15]. While the effects of anillin depletion on anchorage-independent cancer cell growth appear to be consistent, conflicting results have been reported regarding its influence on anchorage-dependent cell proliferation. Several studies that utilized either siRNA or shRNA anillin knockdown observed significant growth inhibition of anillin depleted breast, pancreatic, liver, prostate and bladder cancer cells cultured on conventional plastic surfaces [58, 65, 68, 75, 78]. In contrast, stable CRISPR/Cas9-mediated depletion or overexpression of anillin selectively altered their anchorage-independent soft agar growth but had no effect on proliferation of cells attached to plastic [15]. There are several possible reasons for this discrepancy. One reason is the contribution of non-specific cell growth restricting effects of anillin siRNAs or shRNAs that are driven by the disruption of endogenous micro-RNA functions [79]. The other reason is that stable CRISPR/Cas9-edited breast cancer cell lines are capable to compensate for some, but not all functional defects caused by anillin knockout. The idea of functional compensation for a stable loss of anillin expression in cancer cells is supported by the fact that transient anillin depletion resulted in significant cancer cell death [58, 64]. In contrast, breast cancer cells with stable and very steep (more than 90%) anillin knockout did not demonstrate significant decrease in cell viability in vitro [15]. Likewise, while transient loss of anillin expression caused marked cancer cell polyploidy [61, 64, 69], our unpublished data suggest that this phenomenon was less pronounced in stable anillin knockout breast cancer cells.

In agreement with the described in vitro data, anillin depletion potently inhibits tumor growth in vivo. This tumor growth inhibition was observed in studies that

involved either orthotopic or subcutaneous injection of anillin-depleted breast, bladder, pancreatic, or liver cancer cells into immunocompromised mice [15, 58, 65, 68, 69]. Importantly, the tumor-suppressive effects of anillin depletion in vivo were obvious not only in the 'preventive' mode of anillin depletion prior to tumor development, but also in the 'therapeutic' mode when downregulation of anillin expression was able to reverse the development of already established tumors [69]. These exciting data highlight anillin inhibition as an attractive anti-cancer therapeutic strategy. An open question remains about whether anillin overexpression alone is sufficient to drive tumor development in vivo. While this possibility has not been extensively investigated, our recent study provides some supportive evidence. Specifically, we reported that MCF10AneoT breast cancer cells with stable overexpression of the N-terminal GFP-tagged anillin rapidly truncate the overexpressed protein, retaining approximately 40 kDa N-terminal anillin fragment [15]. Overexpression of this truncated anillin was sufficient to accelerate soft agar growth of breast cancer cells in vitro and to stimulate primary tumor growth after orthotropic cell injection in vivo [15], indicating that the N-terminal part of the molecule is essential for the tumor-promoting activities of anillin in breast cancer cells.

Despite a large body of evidence that implicates anillin in driving tumor growth and proliferation, the underlying mechanisms remain poorly investigated. Surprisingly, little is known about the effects of anillin overexpression on cancer cell division. Depletion of this protein results in a common cytokinesis failure, which is not specific for cancer cells. Anillin overexpression in lung adenocarcinoma cells was shown to stimulate DNA synthesis [64]. Paradoxically, increased anillin level could also lead to a defective cytokinesis, such as attenuated abscission, as it was shown for anillin-overexpressing HeLa cells [35]. Therefore, two different outcomes of the increased anillin level in cancer cells could be envisioned. One is acceleration of mitosis and the other is defective cytokinesis, which leads to aneuploidy. Both outcomes could have tumor-promoting effects via either enhanced cancer growth, or aneuploidy-driven DNA damage and chromosomal instability [4]. It should be noted that the tumor growth accelerating activity of anillin could be unrelated to its direct regulation of cell division and may be due to modulation of the phenotypic plasticity of cancer cells that is discussed in subsequent chapters.

Anillin as regulator of cancer cell migration and metastasis

In addition to its tumor growth-promoting activity, anillin is known to be a potent stimulator of tumor cell migration in vitro. The pro-motile effects of anillin have been demonstrated by several commonly used techniques, such as scratch wound healing, Boyden Chamber migration and Matrigel invasion assays, in a variety of cancer cells [15, 57, 65, 68, 78] and in non-tumorigenic fibroblasts and podocytes [26, 51, 64]. While little is known about anillin-mediated control of tumor metastasis in vivo, a recent study suggests that anillin plays a role in this process. Thus, anillin-depleted breast cancer cells show decreased metastatic spread into the lungs, liver, kidney and lymph nodes in mice [15]. Interestingly, such pro-migratory and pro-metastatic activities of anillin appear to be unrelated to its tumor growth-promoting ability [15]. Several molecular mechanisms have been suggested to explain the effects of anillin on cell motility in vitro. Thus, anillin could regulate breast cancer cell migration by altering cell-extracellular matrix (ECM) adhesions [15]. Loss of anillin in breast cancer cells substantially increases their ECM attachments, thereby attenuating efficient cell motility. Hyperadhesiveness of anillin-depleted cancer cell does not reflect specific alteration of adhesion-related signaling but is associated with assembly of prominent actomyosin stress fibers [14, 15]. Furthermore, enhanced ECM adhesion and stress fiber induction in cancer cells cannot be explained by a direct scaffolding activity of anillin, since in these cells it is exclusively localized in the nuclei [14, 15]. These data suggest that anillin can affect the organization of actin cytoskeleton remotely, via a yet-to-be-defined signaling pathway(s). Other studies conclude that anillin promotes cell migration via direct binding to cytoskeletal regulators. For example, a mutant FSGS-related form of anillin that increases motility of human podocytes demonstrates defective interactions with actin filaments and the actin filament capping protein, CD2AP [26, 51]. In *C. elegans*, anillin promotes neuronal migration by accumulating at the cell cortex and stabilizing actin filaments at the protruding leading edge [52]. Overall, published data indicate that anillin can regulate motility and intercellular adhesions of normal and cancer cells by acting either locally, as cytoskeletal/membrane scaffold at the cell cortex [48–50, 52], or remotely, by regulating intracellular signaling from the nucleus (Fig. 2) [14, 15]. Future studies are required to reconcile these distinct modes of anillin actions.

Anillin as regulator of cancer cell stemness and differentiation

Recent studies revealed an unexpected complexity of anillin functions in cancer cells that is related to the cellular phenotypic plasticity and regulation of cancer stem cells. Thus, upregulation of anillin expression markedly enhances the self-renewal potential of epithelial-type MCF10AneoT cells, whereas loss of anillin decreases stem/progenitor properties of mesenchymal-type MDA-MB-231 and BT549 breast cancer cells [15]. Remarkably, loss of anillin expression in mesenchymal type breast cancer cells induces their

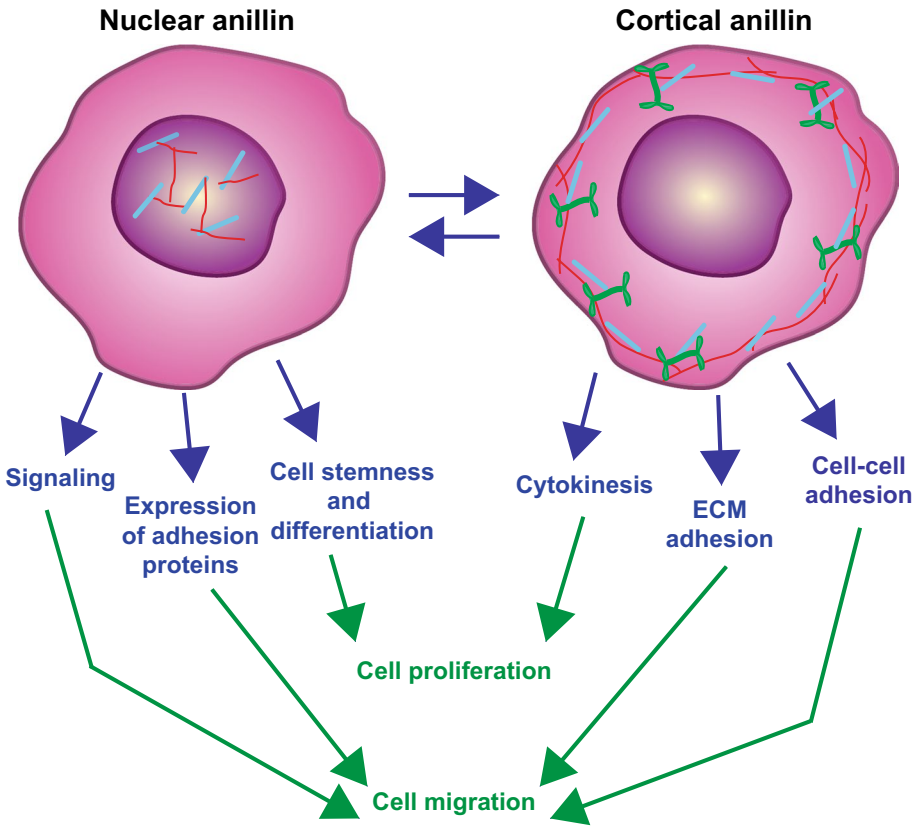

Fig. 2 Cellular mechanisms that mediate tumor-promoting functions of anillin. The diagram shows a hypothetic dual role of anillin in tumor development. This protein could function as either a peripheral scaffold that regulates remodeling of the cell cortex in proliferating and migrating cells or serve as a nuclear transcriptional regulator that promotes stemness and accelerates pro-metastatic signaling in cancer cells. Light blue rods, anillin; red lines, actin filaments; green lines, non-muscle myosin II

trans-differentiation into basal-like epithelia cells, which is manifested by upregulation of basal epithelial cell markers, such as low molecular weight keratins, E-cadherin and P-cadherin [15]. Similarly, transient downregulation of anillin expression in poorly-differentiated A549 and PC9 lung adenocarcinoma cells triggers a classical mesenchymal-to-epithelial transition, accompanied by the increased expression of E-cadherin and decreased expression of mesenchymal markers, N-cadherin and vimentin [72]. The described modulation of cell stemness and differentiation can explain key functional effects of anillin knockout and overexpression, such as modulation of anchorage-independent cell growth in vitro and tumor xenograft development in vivo (Fig. 2). Furthermore, the mesenchymal–epithelial differentiation is likely to be responsible for the suppressed motility of anillin-depleted breast cancer cells. Indeed, one of the crucial events of such trans-differentiation is upregulation of E-cadherin level, which is a known suppressor of cancer cell migration and metastasis [80]. Consistently, siRNA-mediated downregulation of E-cadherin expression was shown to be sufficient to reverse the attenuated motility of anillin-depleted breast cancer cells [15].

The emerging roles of anillin in modulating the phenotypic plasticity of cancer cells may reflect a broader ability of this protein to regulate cell stemness. For example, high anillin expression is a characteristic feature of rapidly proliferating pluripotent stem cells in mouse embryos [81], somatic stem cells in *Drosophila* testes, [82] and neuronal precursors in zebrafish retina [83, 84]. On the other hand, a dramatic decrease of anillin expression was observed in senescent primary human fibroblasts and cervical carcinoma cells [85, 86]. While molecular mechanisms underlying the effects of anillin on stem cells remain unknown, they could be associated with expressional regulation of the stem cell-specific transcriptional program. Indeed, our recent RNA sequencing (RNAseq) analysis reveals downregulation of several transcription factors essential for stem cell biogenesis, including Ovol2, TFCP2L1, FOXK1, PBX1, TBX18 and SOX9 in anillin-deficient breast cancer cells [15]. Determining which of these transcriptional factors are involved in anillin-dependent regulation of cancer cell stemness and differentiation remains an important question for future investigations.

Anillin controls global transcriptional programming of cancer cells

While predominantly nuclear localization of anillin in different cancer cells has been extensively documented (Table 1), functions of nuclear anillin in tumorigenesis has been surprisingly underappreciated. A traditional view of the anillin roles in cancer cells implies that overexpression of this

protein results in its leaking into the cytoplasm, where anillin plays tumorigenic roles by promoting cell division and migration [13, 87]. While function of cytoplasmic/cortical anillin is likely to be important for tumor growth and metastasis, such function is insufficient to account for the anillin-dependent regulation of the phenotypic plasticity and stemness of cancer cells. Furthermore, actions of nuclear, not peripheral anillin could explain the results of recent studies demonstrating large-scale perturbations of gene transcription caused by anillin depletion in cancer cells. Indeed, published whole-genome RNAseq or microarray analysis of breast, pancreatic and bladder cancer cell lines reports either expressional downregulation or upregulation of tens-to-hundreds different genes following knockout or knockdown of anillin [15, 57, 65, 68]. A gene ontology analysis performed in these studies reveals dysregulation of multiple molecular pathways in anillin-deficient cancer cells (Table 2). For example, loss of anillin causes transcription dysregulation of molecular pathways related to cell motility, ECM adhesion and actin cytoskeleton in pancreatic cancer cells [57], which is consistent with the altered migration, adhesion as well as cytoskeletal remodeling caused by anillin depletion in these cells. Additionally, in breast cancer cells, anillin knockout triggers transcriptional upregulation of the pathways linked to epithelial and epidermal cell differentiation and keratinization, which correlates well with the observed mesenchymal to basal epithelial trans-differentiation of these cells [15]. The non-canonical molecular pathways affected by anillin depletion involve regulation of RNA polymerase II transcription in breast cancer cells [15], Toll-like receptor and cytokine signaling in bladder cancer cells [68] and DNA replication, RNA nuclear export and protein translation in pancreatic cancer cells [65]. Interestingly, possible anillin functions in regulating protein translation which was alluded by the microarray analysis was recently confirmed by a genome-wide functional siRNA screen in human cells

Table 2 Molecular pathways affected by anillin depletion in cancer cells

Type of cancer	Gene expression analysis	Pathway analysis	References
Breast cancer (MDA-MB-231 cells)	RNA sequencing	Upregulated pathways (GO analysis): Epithelium development Epithelial cell differentiation Keratinization Programmed cell death Tissue development Downregulated pathways: Regulation of transcription by RNA polymerase II Regulation of nucleic acid-templated transcription Regulation of multicellular organismal development	[15]
Pancreatic cancer (PANC-1 cells)	Microarray analysis	Downregulated pathways (KEGG analysis): Focal adhesions ECM-receptor interactions Regulation of actin cytoskeleton Leukocyte transendothelial migration Complement and coagulation cascade	[57]
Pancreatic cancer (BxPC-3 cells)	Microarray analysis	Upregulated pathways: Poly(A) RNA binding Cadherin binding involved in cell–cell adhesion Nucleosomal DNA binding ATP-dependent RNA helicase activity Structural constituent of ribosome Nuclear localization sequence binding DNA helicase activity Translation initiation factor activity Ran GTPase binding ATP binding Histone binding	[65]
Bladder cancer (J82 cells)	Microarray analysis	Upregulated pathways: Cytokine–cytokine receptor interactions Jak-STAT signaling pathway Toll-like receptor signaling pathway PI3K-Akt signaling pathway Salmonella infection TNF signaling pathway Ovarian steroidogenesis	[68]

[88]. This screen identified anillin as essential regulator of the global protein synthesis by mechanisms involving ribosomal pre-RNA processing and nucleoli biogenesis [88]. The diversity of signaling pathways and molecular processes altered by anillin depletion indicates that currently, there is a very limited understanding of the functional activity of this protein in cancer cells and many important anillin functions remain to be discovered.

It has been hypothesized that anillin-dependent regulation of the transcriptional program of cancer cells could involve control of the polymerization and functions of nuclear actin [15]. Nuclear actin is a well-known regulator of gene transcription that binds to and modulates the activity of RNA polymerases (RNAP) and serves as a structural component of the major chromatin remodeling complexes [89–91]. The actin-dependent regulation of gene expression is known to be dependent on the polymerization status of nuclear actin and is regulated by several actin-binding proteins present in the nuclei [90–94]. Since anillin binds to actomyosin filaments and is markedly enriched in the nuclei of cancer cells, we proposed that it can modulate nuclear actin interactions with essential components of the transcriptional machinery such as RNAP. This could lead to the altered expression of different subsets of genes, including those regulating cancer cell stemness and differentiation. While there is no direct proof of these proposed interactions and/or the functional interplay between anillin and nuclear actin, our study provides indirect evidence in support of this idea [15]. For example, the RNAseq analysis reveals downregulation of pathways involving RNAPII-dependent transcription in anillin-deficient breast cancer cells (Table 2). Furthermore, a truncated N-terminal fragment of anillin with the preserved actin-binding site selectively accumulates in the nuclei of breast cancer cells and stimulates cancer cell growth and motility [15]. A recently discovered novel physicochemical feature of anillin could be essential for its nuclear function in transcriptional reprogramming of cancer cells. Specifically, yeast anillin was found to undergo a phase separation to form liquid droplets [95]. Phase separation is an emerging mechanism that regulates gene expression by condensing RNAPII, transcription factors and chromatin complexes into distinct and dynamic nuclear domains [96, 97]. It is tempting to speculate, therefore, that the described transcriptional activity of anillin could be linked to its ability to form different liquid-like nuclear protein phases. Further studies will help to establish the precise roles and mechanisms of nuclear anillin-dependent regulation of tumor development.

Mechanisms that regulate anillin expression and function in cancer cells

Anillin is a very dynamic protein, which expression and localization markedly fluctuates at different stages of the cell cycle [11, 13, 21]. Several mechanisms that control anillin expression on the transcriptional and posttranslational stages have been identified in model cells and organisms and it is likely that dysregulation of these mechanisms contributes to anillin overexpression in cancer cells. For example, a recent study identified anillin as a major downstream transcriptional target for the androgen receptor in an in vitro model of castration resistant prostate cancer [98]. The androgen receptor was shown to form a complex with the octamer transcription factor 1 (OCT1) and bind to the enhancer/promoter region of anillin gene driving its transcription. Downregulation of either AR, or OCT1 markedly reduced anillin mRNA expression in prostate cancer cells [98]. In silico analysis of gastric cancer suggested that anillin expression could also be regulated by the Wnt/β-catenin-dependent signaling [63]. This study observed strong positive correlation between anillin and β-catenin expression in gastric cancer mRNA expression profiles. In addition, transcription factor analysis revealed the presence of binding sites for the Wnt/β-catenin-dependent TCF transcription factor in the promoter of the anillin gene [63]. It is noteworthy that experimental data to demonstrate causal roles of the Wnt/β-catenin signaling in regulating anillin expression are lacking. Interestingly, anillin expression was found to be upregulated in cancer-associated fibroblasts, driven by the elevated activity of a YAP transcriptional factor [99]. In this system, anillin appears to participate in a feed-forward self-reinforcing loop that promotes actomyosin contractility and induces extracellular matrix stiffening, thereby further promoting YAP activation [99].

An alternative reason for anillin upregulation in cancer could be linked to defects in the mechanisms that normally repress expression of this protein. Such mechanisms involve a number of antitumor micro-RNAs (miRs). Indeed, several recent studies described multiple miR species, miR-15a, miR-130, miR-217 and miR-497, that interact with the 3′ UTR region of anillin mRNA and block its expression [57, 58, 74, 100]. The decreased expression of these anillin-blocking miRs was implicated in the increased expression of anillin and its tumorigenic activity in pancreatic adenocarcinoma, nasopharyngeal carcinoma and hepatocellular carcinoma cells [57, 58, 74, 100].

The cellular level of anillin could also be regulated by its protein degradation. This mechanism is evolutionary conserved from yeasts to mammals and involves anillin ubiquitination by different ubiquitin ligases with subsequent proteasomal degradation. In mammalian cells, ubiquitin–proteasomal degradation of anillin is driven by its interactions with E3 ubiquitin ligase, the anaphase-promoting complex/cyclosome (APC/C), during cell cycle progression [21]. APC/C binds to the destruction box (D-box) motifs in the N-terminal part of the protein (Fig. 1) leading to anillin degradation during mitotic exit. Similarly, in fusion yeasts,

an anillin-like protein, Mid2p is also targeted to ubiquitin-dependent degradation via interactions with a Skp1/Cdc53/F-box ubiquitin ligase complex [101]. Since APC/C activity could be inhibited in different types of neoplasia [102], such inhibition could contribute to the increased level of anillin protein observed in cancer cells.

Another level of regulation of anillin cellular distribution and function could be imposed by its posttranslational modification. Anillin is known to be phosphorylated during mitosis in yeast [103, 104] and mammalian cells [25, 105]. Furthermore, anillin phosphorylation is essential for its localization and function during cytokinesis [103–105]. In HeLa cells, anillin is phosphorylated on multiple serine and threonine residues positioned throughout the entire protein molecule. This phosphorylation could be mediated by different mitotic kinases, such as polo-like kinase 1, Aurora B and cyclin-dependent kinase 1 [105]. The possible roles and mechanisms of anillin phosphorylation in cancer remain largely unknown. It was shown to be highly phosphorylated in lung adenocarcinoma cells, in a PI3K/AKT-dependent fashion [64]. Furthermore, serine phosphorylation of anillin occurs in breast cancer cells exposed to estrogen receptor agonists, although the functional consequences of such phosphorylation has not been investigated [106]. In addition to phosphorylation, anillin can also undergo reversible acetylation in mitotic cells on unspecified lysine residues [107]. Although it has been speculated that anillin acetylation may affect the assembly of multiprotein complexes during cytokinesis, experimental proof of this hypothesis is still lacking.

Conclusion

A large body of clinical evidence demonstrates marked overexpression of anillin in many types of solid tumors that significantly correlates with poor prognostic outcomes in cancer. These data could signify anillin as a potential cancer biomarker and a crucial molecular driver of tumorigenesis, however, more research is needed to establish a direct relationship between the anillin levels and tumor progression. Importantly, recent studies revealed unexpected complexity of anillin-dependent regulation of cancer cell growth and motility. As a multivalent cytoskeletal scaffold, anillin directly controls cytokinesis of rapidly-dividing cancer cells and cytoskeletal remodeling of migrating metastatic cancers. In addition, anillin serves as an emerging regulator of the transcriptional program essential for cancer cell stemness and differentiation. Studies of anillin functions and regulation in cancer are currently gaining momentum in the cancer research field, and future investigations may soon provide significant novel insights into the mechanisms of anillin actions in tumor growth and metastasis that would allow targeting this oncogenic protein for the development of novel anti-cancer therapies.

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