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Mini-review

p27^{Kip1} and human cancers: A reappraisal of a still enigmatic protein

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ABSTRACT

p27^{Kip1} is a cell cycle regulator firstly identified as a cyclin-dependent kinase inhibitor. For a long time, its function has been associated to cell cycle progression inhibition at G1/S boundary in response to anti-proliferative stimuli. The picture resulted complicated by the discovery that p27^{Kip1} is an intrinsically unstructured protein, with numerous CDK-dependent and -independent functions and involvement in many cellular processes, such as cytoskeleton dynamics and cell motility control, apoptosis and autophagy activation. Depending on the cell context, these activities might turn to be oncogenic and stimulate cancer progression and metastatization.

Nevertheless, p27^{Kip1} role in cancer biology suppression was underscored by myriad data reporting its down-regulation and/or cytoplasmic relocalization in different tumors, while usually no genetic alterations were found in human cancers, making the protein a non-canonical oncosuppressor.

Recently, mostly due to advances in genomic analyses, *CDKN1B*, p27^{Kip1} encoding gene, has been found mutated in several cancers, thus leading to a profound reappraisal of *CDKN1B* role in tumorigenesis. This review summarizes the main p27^{Kip1} features, with major emphasis to its role in cancer biology and to the importance of *CDKN1B* mutations in tumor development.

Introduction

p27^{Kip1} (hereinafter reported as p27) was identified as a protein able to bind and inhibit the activity of cyclin E/cyclin-dependent kinase 2 (CDK2) complex [1–3]. In 1994, the gene encoding human p27 (*CDKN1B*) was cloned [4] and mapped to chromosome 12p13 (Fig. 1) [5]. The protein shares significant sequence homology with the other two members of the Cip-Kip CDK inhibitor family, p21^{Cip1} and p57^{Kip2}. p27 orthologues have been identified in nine mammalian species, and in *Xenopus laevis* [6], yeast [7] and *Arabidopsis thaliana* [8].

For several years, the capability to inhibit the cyclin/CDK complexes has remained the exclusive p27 function. However, depending on the cyclin/CDK heterodimer targeted, the interaction shows remarkable differences. While p27 role in inhibiting cyclin E(A)/CDK2, particularly at G1/S boundary of the cell cycle, has been well characterized, p27 interplay with cyclin Ds/CDK4(6) is more complex. First, although p27 inhibits with a similar efficiency cyclin D/CDK4 and cyclin A/CDK2, significant changes in the enthalpy/entropy balance associated to the binding were reported [9]. Second, the inhibition is valuable in quiescent cells, while it is ineffi-cacious in proliferating cells [10]. Moreover, in growing cells p27 induces the assembly of cyclin Ds/CDK4(6) complexes and their nuclear translocation [10]. Few data exist on the interaction between p27 and cyclin B/CDK1, suggesting that p27 inhibits the complex in CDK2-ablated mice [11,12]. Accordingly, in p27$^{-/-}$ animals, an increase of CDK1 activity was observed [11,12].

Numerous p27 protein partners have been identified in the last years, arguing for a p27 role in several CDK-unrelated processes. Some of the reported interactors appear of peculiar interest from a physiological/pathological point of view, including: Jab1, CRM1, COP9, Spy, RhoA, Rac, stathmin, Grb2, 14-3-3, Jak2, HIPK2, citron K and HSC70 (reviewed in [13–16]). By binding to them, p27 modulates cell adhesion and motility, mitotic spindle, cell death processes, transduction pathways and transcription-regulating

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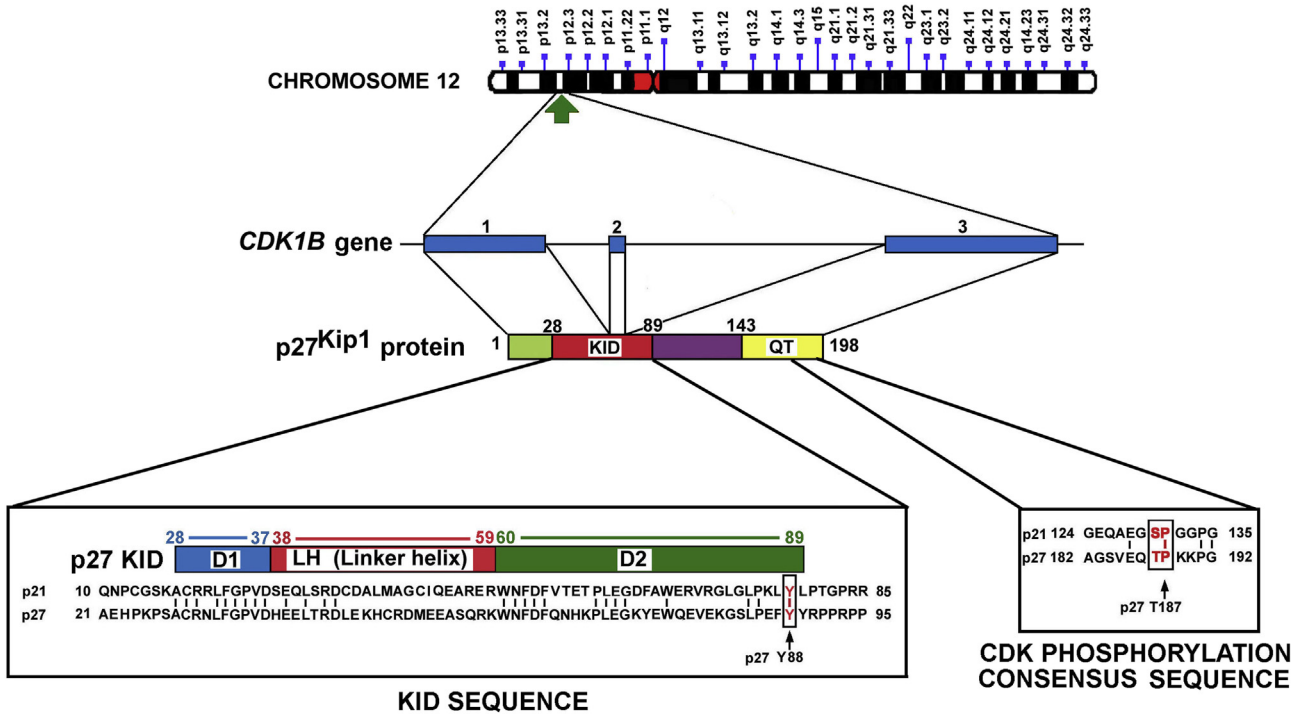

Fig. 1. Genomic *CDKN1B* organization and p27^{Kip1} protein domain structure. The figure reports (from the top to the bottom), the genomic localization of *CDKN1B* (chromosome 12p13), the exon-intron organization of the gene and the domain structure of p27^{Kip1}. Two sequences of the human p27^{Kip1} (hp27) are reported in detail and compared to the corresponding sequences of human p21^{Cip1} (hp21), namely the KID (Kinase Inhibitory Domain) and the *consensus* domain phosphorylated by active CDK2 and acting as phosphodegron. KID is modular and includes the cyclin-binding sub-domain (a region termed D1), the CDK binding sub-domain (a region termed D2) and a 22 residue sub-domain (a beta-structure defined as LH) that joins the subdomain D1 to subdomain D2. Initially, a short sequence of D1 KID binds the cyclin surface. Then, the LH subdomain contacts the cyclin and CDK surfaces and allows D2 subdomain to adopt an extensive secondary structure able to make several interactions with the CDK catalytic subunit. In particular, Y88 that is localized approximately at the C-end of the KID, after the interaction of p27 with the cyclin A(or E)/CDK2 is accommodated in the ATP-binding pocket of Cdk2, thus hampering the kinase activity.

complex [13–16]. The next paragraph briefly summarizes the structure and roles of p27.

Structure, metabolism and functions of p27

p27 structure

Although p27 has been the focus of extensive investigations, several structural/functional features of the protein are still poorly understood. Foremost obstacles to their elucidation stem from p27 structure that is, in large part, intrinsically unfolded [17,18]. Indeed, p27 belongs to the Intrinsically Unstructured Proteins (IUPs), a protein family characterized by the lack of stable secondary/tertiary structure and by the ability to fold only upon encountering putative interactors [18,19]. This p27 "plasticity" strongly enhances the spectrum of its activities [18,19]. In general, the binding of an IUP to its targets is strongly affected by post-synthetic modifications (PTMs) that enable rapid switches among different IUP structural conformations. This potentiality has been reported for key oncogenic proteins such as CBP/p300, p53, BRCA1, CREB, HIFα, PTEN, c-Myc, I*k*B, and others [19], and obviously increases enormously the importance of p27 PTMs in addressing the protein towards specific ligands.

p27 sequence analysis allows the identification of two major regions (Fig. 1). The N-end terminus contains a highly conserved region (residues 28–89) called "kinase inhibitory domain" (KID) [20,21] that is able to inhibit cyclin/CDK activity. Initially disordered, the KID assumes a stable structure only upon binding to a CDK complex [see also Fig. 1 legend] [20,21]. In inhibiting cyclin A(E)/CDK2, p27 interacts first with the cyclin through the specific KID D1 sub-domain; the event induces a conformational change that allows p27 adjustment to and inhibition of CDK2 [20]. Specifically, two main different mechanisms appear to engender the catalytic inhibition, namely p27: i) hinders ATP and substrate recognition/binding site(s), and ii) probably prevents CDK activating phosphorylation(s) [22–25].

The p27 C-end region (residues 105–198) has been poorly characterized. It is subjected to different PTMs and is able to bind several proteins in different cellular compartments (see next paragraph) [13,14]. Although lacking a definite folding, some structure have been recently reported for cyclin A/CDK2-bound p27, in that the initial part of the C-end region (aa 110–140) forms an angle perpendicular to cyclin A/CDK2 surface, forcing p27 to assume a highly extended conformation [17].

p27 metabolism

p27 content is regulated by transcriptional and post-transcriptional mechanisms [16]. The transcriptional modulation probably plays a minor role in p27 level control. It involves several transcription factors (TFs) including Myc, Foxo, and Menin [16]. Myc down-regulates p27 acting at different levels, namely reducing *CDKN1B* transcription [26–28], up-regulating p27 transcript-targeting microRNAs [29,30], and increasing p27 degradation [31,32]. Some members of the Forkhead box O proteins family (Foxo4, Foxo3a, Foxo1a) induce *CDKN1B* transcription, leading to arrest of proliferation at G0/G1 boundary [28,33]. Menin (encoded by *MEN1* gene) is a TF whose mutations are responsible for multiple endocrine neoplasia type 1 (MEN1) [34]. Menin has been described to interact with *CDKN1B* gene promoter enhancing its expression by histone methyltransferase activity modulation [35].

Despite the identification of a transcriptional regulation, p27 levels are mainly controlled through protein turnover/degradation. At least two removal pathways have been deeply characterized. One is required for S phase entry and occurs at nuclear level. It involves T187 phosphorylation (by active CDK2) that creates a phosphodegron for ubiquitination and proteasome degradation [36,37].

Particularly, phosphorylated T187 side chain of p27 is recognized by the ubiquitin ligase complex SCF-Skp2 (Skp1-Cul1-Rbx1-Skp2), where Skp2 represents the F-box protein responsible for the specific recognition of the substrate [38–40]. The binding to this E3 complex also requires the accessory protein Cks1 [40].

Since p27 inhibits cyclin E(A)/CDK2 activity, the involvement of CDK2 in p27 degradation appears, at a first glance, quite obscure. However, the order of events that shifts p27 from being a CDK2 inhibitor to become a substrate has been clarified (Fig. 2) [21–23]. In brief, specific p27 KID regions remain flexible after the binding with cyclin/CDK2 complex, allowing non-receptor tyrosine kinases to phosphorylate specific p27 tyrosine residues (mainly Y88) [21–23,41]. In turn, this causes the ejection of the phosphoY residue from the CDK2 catalytic pocket and, consequently, the kinase activation. Finally, p27 C-terminus flexibility allows T187 phosphorylation by activated CDK2. Therefore, it has been proposed that p27 represents a "conduit" for communicating a proliferative

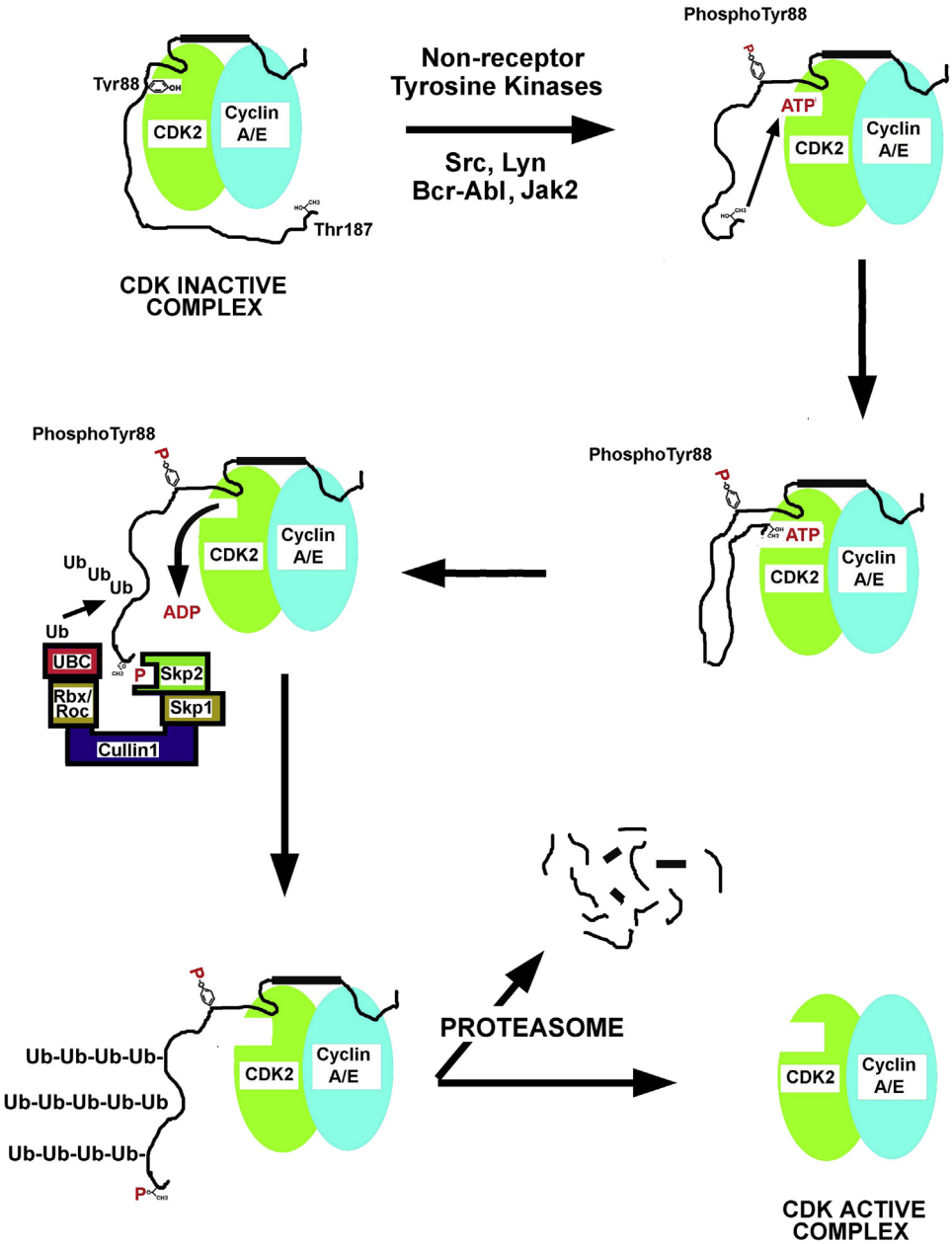

Fig. 2. Mechanism of activation of p27^{Kip1}/cyclin/CDK by non-receptor tyrosine kinases. Starting from top on the left. Active non-receptor tyrosine kinase (like Src, BCR-Abl, Lyn, Jak2 and others) phosphorylates Y88 that is then ejected from the CDK2 catalytic pocket. Subsequently, cyclin A(E)/CDK2 phosphorylates T187 p27 creating a phosphodegron for SCF/Skp2 E3 complex. Finally, polyubiquitinated p27 is degraded by the proteasome complex. Ub, ubiquitin.

stimulus through a PTM. Among several other p27 degradation mechanisms described, only one has been characterized in details [42,43]; it occurs in the cytosol and involves the so-called Kip1 ubiquitination-promoting complex (KPC) [42,43]. Importantly, it allows p27 degradation when cells reenter the cycle from quiescence. However, some details are still uncertain, including the interplay among KPC activity, p27 nuclear/cytosol shuttling and p27 PTMs.

p27 levels and activities also depend on the protein cellular localization. As a matter of fact, p27 interactors and degradation appear strictly dependent on compartmentalization. p27 sequence shows a bipartite nuclear localization signal (NLS) and a putative nuclear export signal (NES) (Fig. 3) ([13] and references therein). Based on Literature, p27 shuttling from cytosol to nucleus and *vice versa* seems extremely intricate. In brief, after its synthesis, p27 might be transported into the nucleus where it interacts with (and generally inhibits) different cyclin/CDK complexes. Nuclear entry might be prevented by cytosolic T157 or T198 phosphorylation [44–46]. The protein sequestered in the cytoplasm might bind several interactors (reviewed in Refs. [13–16]) or, in G0/G1, might be degraded through KPC activity [42,43]. On the other hand, phosphoT157/phosphoT198-p27 (pT157/pT198-p27), as previously mentioned, participate to cyclin D/CDK4(6) complex assembly and nuclear translocation [47]. Nuclear p27 can follow at least two different fates, i.e. binding cyclin/CDKs or being phosphorylated in S10. pS10-p27 interacts with Jab/CRM1 complex and return to the cytosol [48–50]. A fraction of p27 bound to cyclin/CDK complex might be phosphorylated in Y88/T187 and degraded. Y88p27 phosphorylation is also necessary for activating cyclin D/CDK4(6) bound to p27 [9,10,47]. This description of p27 shuttling/metabolism represents an attempt to summarize some of the data reported in Literature and appears, in part, as an inexplicable labyrinth. However, in our view, it is mostly dependent on the experimental model(s) and thus, should be considered with caution.

p27 functions

As anticipated before, numerous p27 functions, not related to CDK inhibition, have been described. For the sake of brevity, we will discuss only those mainly associated to cancerogenesis. An exhaustive picture of p27 roles has been reported elsewhere [13–16].

One well-documented cytosolic p27 role is the regulation of cytoskeleton assembly/disassembly and, in turn, of cell shaping and movement. Whether the protein accumulation promotes or inhibits cell contractility is not plain and probably dependent either on cell phenotype or on abundance/scarcity of p27 specific interactors. In mammals, stress fiber dynamics is central for cell

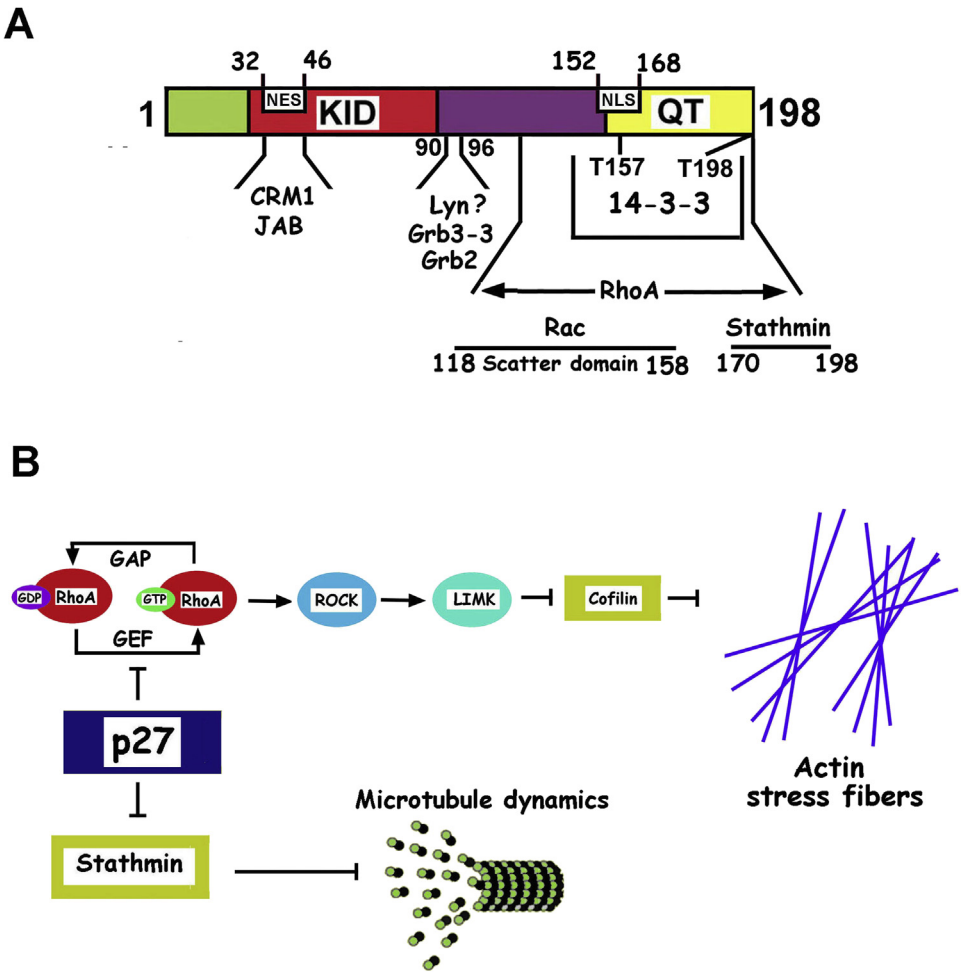

Fig. 3. Main p27^{Kip1} interactors and p27^{Kip1} effect on cell motility. Panel A: the image shows the localization of NES (nuclear exporting sequence) and NLS (nuclear localization signal). In addition, the main p27 interactors are reported. **Panel B**: effect of p27 on cell growth. The protein is able to inhibit the activation of RhoA and, by a pathway involving ROCK/LIMK, to activate cofilin. Finally, active cofilin disassembles actin filaments, reduces stress fiber stabilization and focal adhesion formation and increases cell motility. On the other hand, p27 also inhibits stathmin, a microtubule-destabilizing protein, causing cell motility reduction.

contractility, representing the basis of cell adhesion, morphogenesis, migration and cancer metastatisation (Fig. 3). Stress fibers are mostly formed by actin and myosin. Actin rapidly polymerizes/depolymerizes, forming fibers of about 10–30 units. RhoA GTPase is a major responsible for actin fiber stabilization. Active RhoA up-regulates a signaling cascade (involving ROCK and LIM kinase) that phosphorylates and inactivates cofilin, an actin filament depolymerizing factor (Fig. 3). In several models p27 inhibits RhoA by preventing its activation [51–55]. Hence, a cytosolic p27 up-regulation reduces stress fiber stabilization and focal adhesion formation, increasing cell motility. Accordingly, Besson et al. demonstrated that in p27-negative MEFs a cell contractility decrease occurs, rescued by p27 restoration [51]. Moreover, Slingerland's group reported that, in WM35 cells, RSK1 phosphorylates p27 on T198, promoting RhoA inhibition and enhancing cell motility [56]. In 2012, it was demonstrated that p27 controls cortical interneuron morphological changes and, particularly, nucleokinesis and neurite branching [57,58]. Molecularly, p27 either inhibits RhoA or acts as a microtubule-associated protein, promoting microtubule polymerization [58]. Finally, a very recent study shows, in T47D breast cancer cells, that progesterone induces, through cSrc/AKT/RSK1 pathway activation, p27 T198 phosphorylation, thus up-regulating p27-associated RhoA activity, and unexpectedly, increasing cell motility [59].

In contrast with the previous data reporting a p27 positive effect on motility, further convincing evidence suggests that p27 up-regulation inhibits cell contractility. Particularly, several studies demonstrate that cytosolic p27 inhibits stathmin, a microtubule-destabilizing protein, causing cell motility reduction (Fig. 3) [60–62]. Accordingly, low cytoplasmic p27 or high stathmin levels correlate with the metastatic phenotype of human sarcomas *in vivo* [60]. Moreover, a recent report showed that p27 and stathmin interaction down-regulates H-Ras/MAPK and cyclin D/CDK4(6) activities [63].

Contradictory pieces of evidence also address p27 role in apoptosis and autophagy modulation [64–77]. In some cancer cell lines, p27 overexpression promotes apoptosis [64–66] while, in other experimental models, it favors survival reducing pro-apoptotic anticancer drug effects [70,71]. Furthermore, it has been shown that caspase 3 cleaves p27 and, *vice versa*, p27 regulates procaspase 3 activation [67,72]. p27 is also involved in autophagy modulation, as suggested by its ability to act as substrate of AMP-activated protein kinase (AMPK), a key energy-controlling enzymatic activity [73]. Recently, p27 has been reported as positively modulating metabolic stress-induced cardiomyocyte autophagy [75]. Further CDK-independent p27 functions have been reported, including transcription control, regulation of mitosis and tissue morphogenesis. For brevity, we address the readers to reviews that discuss these issues in details [13–16,78].

Deciphering p27 post-synthetic modifications

As for all the IUPs, PTMs are essential for p27 commitment and selection of interactors/functions. The PTMs so far identified are mostly phosphorylations, but acetylation, glycosylation, ubiquitination and methylation have also been reported. Human p27 phosphorylations are shown in Fig. 4, and mainly involve the following residues: S10, Y74, Y88, Y89, T157, T187 and T198 [13–16,78] and references therein. Other sporadically described phosphorylated residues are: S12, S83, S140, T170, S178 and S183

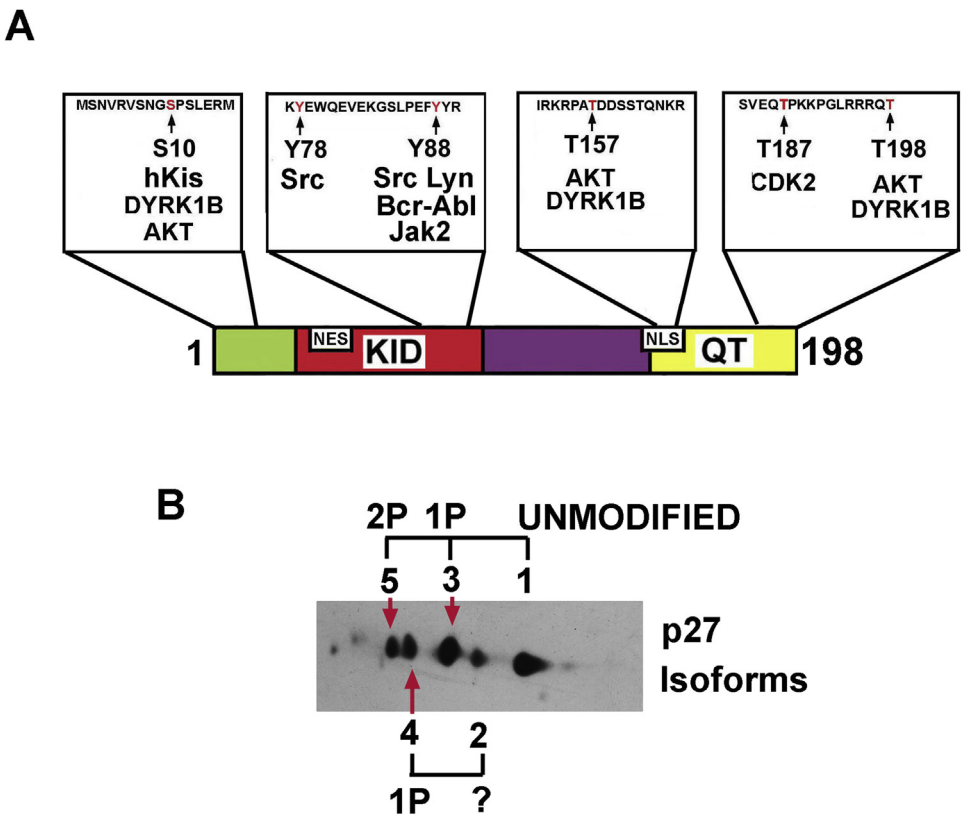

Fig. 4. Main p27^{Kip1} phosphorylation sites and example of p27^{Kip1} bidimensional analyses. **Panel A**: the panel reports the main sites of phosphorylation (S10, Y78, Y88, T157, T187, T198), their *consensus* sequences and the putative kinases responsible for the phosphorylations. **Panel B**: the image shows an example of bidimensional separation of p27 followed by immunoblotting. The sample analyzed is a K562 total cell extract (500 μg protein) **Signal 1** represents the unmodified p27, **signal 3** is the monophosphorylated isoform and **signal 5** shows the biphosphorylated derivative. The **signal 2** is a non-characterized p27 form, while **signal 4** is a monophosphorylated derivative of **signal 2**.

[79–86]. Based on our experience, two major difficulties might be encountered while studying protein phosphorylation, namely: i) the occurrence of isoforms contemporaneously modified in different residues, and ii) the quantitative estimation of each phosphoisoform (i.e. the percentage of each specifically modified protein isoform). In addition, the definitive identification of the kinase(s) responsible for a specific phosphorylation frequently remains elusive, being mainly based on computer-aided search for *consensus* sequences and on *in vitro*/*in vivo* mutagenesis/transfection experiments. The intrinsic p27 lack of structure adds further layers of difficulties to these studies. The most reliable technique for identification of phosphorylated residues is undoubtedly mass spectrometry (MS) analysis. However, the limited p27 cellular content, the complexity of its phosphorylation pattern and, frequently, the numerosity of samples to be analyzed might render MS an unfeasible approach. As MS alternative, we have employed electrophoretic bidimensional separation followed by immunoblotting (defined as 2D/WB) [87,88]. The method was reliable for multiple sample analyses, highly reproducible and favored by the p27 isoelectric point (pI, 6.54) that enhances the phosphorylation effect on pI shift(s). An example of this analysis is reported in Fig. 4 [87,88]. By this approach, we confirmed that almost the totality of the detectable p27 phosphorylation occurs on S10, and demonstrated that: i) the phosphorylation degree and pattern vary independently of total p27 level; ii) nuclear pS10-p27 is mainly bound to cyclin E/CDK2 rather than to cyclin A/CDK2, and iii) nuclear pS10-p27 is more stable than the non-modified p27 since, although participating to CDK ternary complexes, it is scarcely T187 phosphorylated. Conversely, pS10-27 barely binds to CDK1, allowing the hypothesis that the lack of this interaction facilitates mitosis completion. In brief, our data suggested that nuclear p27 follows two almost independent pathways, operating at different rates. One pathway involves tyrosine 88 and threonine 187 phosphorylations and drives the protein towards Skp2-dependent removal. These modifications are clearly assessable only after inhibition of nuclear p27 proteasomal degradation, making difficult their quantitative evaluation [87,88]. The other pathway involves S10 phosphorylation and results in p27 half-life elongation, refinement of CDK targets and nuclear exit. As described before, other p27 PTMs have been reported including: glycosylation on S2, S106, S110, T157 and T198; acetylation on K81 and K134; ubiquitination on K14, K34, K153 and K165, and methylation on R15 (monomethylation) and R134 (dimethylation) [89,90]. S2-O-linked N-acetylglucosamine p27 has been reported to increase in hepatocellular carcinoma, facilitating S10 p27 phosphorylation and its cytosolic relocalization [90]. The glycosylation negatively interferes with the CDK-inhibitory activity, thus favoring cancer proliferation [90]. While p27 ubiquitination is clearly linked to protein removal [91], no definitely data on acetylation and methylation are available.

p27 and cancer

Unregulated cell growth is a major hallmark of cancer. Therefore, much attention has been paid to levels and activities of proteins modulating cell cycle progression, including p27. Accordingly, human tumors with an abnormal p27 metabolism/localization show diminished overall and progression-free survival, and scant response to therapy.

The main cancer-associated mechanisms of p27 alteration, initially reported, were: i) reduction of the protein content (due to increased proteolysis or down-regulated mRNA translation); ii) cytosolic p27 mislocalization, and iii) p27 sequestration in cyclin D/CDK4(6) complexes (reviewed in Refs. [14,78,92–118]). Particularly, several tumors show decreased detectable p27 levels, including colon, lung, prostate, breast, esophageal carcinomas, head and neck cancers, melanomas, gliomas/astrocytomas, Barrett's associated adenocarcinomas and hematological tumors ([14,78,92–118] and references therein). The reduced levels were explained by an upregulation of the protein degradation rate, since no changes of p27 transcripts were generally found ([14,78,92–118] and references therein). The accelerated p27 removal is frequently associated to increased Skp2 and Cks1 levels, the ubiquitin ligase components required for nuclear p27 degradation and dominant oncogenic proteins [29,119–128]. It has also been reported that in some tumors (hepatocellular and thyroid carcinomas, chronic lymphocytic leukemia, glioblastomas, pancreatic adenocarcinomas) p27 mRNA down-regulation occurs as consequence of miR-221 and miR-222 activities, conceivably due to Myc up-regulation [29,30,129–134]. In addition, p27 decrease might be due to enhanced Src (or Src-like kinases) activity that, as reported before, phosphorylates p27 in Y78/88 and targets the protein for destruction [22,23].

Several cancers (melanomas, colorectal, esophageal and breast carcinomas, Barrett's associated adenocarcinoma) show p27 cytoplasmic accumulation as consequence of T198 (T157) phosphorylation that prevents p27 entry into the nucleus [44–46]. T198/T157 phosphorylation could be due (not exclusively) to Akt/PKB, a kinase recurrently activated in various tumors [135].

For a long time, p27 has been considered a non-canonical oncosuppressor protein, since no *CDKN1B* mutations were found in cancers. Recently, however, the availability of mouse cancer models, the development of next-generation sequence techniques and of genome/exome-wide association analyses has led to the identification of *CDKN1B* mutations/haploinsufficiency in several cancers and to an unexpected breakthrough on *CDKN1B* role in tumorigenesis. In the original two-hit Knudson hypothesis, both the alleles of a tumor suppressor gene (TSG) must be inactivated to turn off its suppressive action and promote malignant transformation [136]. Conversely, investigations demonstrated that numerous TSGs drive cancer even when expressed at reduced dosage (the so-called haplo-insufficiency) [137]. *CDKN1B* homozygous ablated mice show a grossly normal development, but are larger than wild-type mice, with hyperplasia or adenoma of intermediate hypophysis lobe and female infertility (all phenotypes linked to cell proliferation abnormality) [138]. Heterozygous *CDKN1B*$^{+/-}$ animals, when irradiated or challenged with chemical carcinogens, develop various tumors, including intermediate lobe pituitary adenomas, intestinal, gonadal, adrenal and lung tumors [138]. The finding suggests that p27 haploinsufficiency might be an important factor favoring cancer development. In humans, numerous studies have now demonstrated that *CDKN1B* mutations are associated to, and probably are responsible for, human cancers. Table 1 reports an updated list of cancer-associated *CDKN1B* mutations so far identified [139–160]. Following, we will describe human neoplasias in which *CDKN1B* mutations have been found.

Multiple endocrine neoplasias (MEN)

MEN are autosomal dominant disorders characterized by neoplasias implicating at least two endocrine tissues [161]. Two MEN forms (MEN1 and MEN2) have been genetically and clinically clearly characterized [161]. MEN1 is due to the *germline* loss-of-function of *MEN1* [161] resulting in the development of primary hyperparathyroidism, parathyroid adenomas, or other endocrine tissue tumors, such as pancreatic and anterior pituitary adenomas [161]. MEN2 appears in two clinical forms, MEN2A and MEN2B, both caused by germline activation of *RET* gene [162]. The clinical features of these syndromes are reviewed in detail in Ref. [161,162]. In 2006, Pellegata et al. demonstrated that MENX rats (an animal

Table 1
CDKN1B mutations in human cancers.

Mutation	Protein change	*CDKN1B* status	Reference
Multiple Endocrine Neoplasia			
5′-UTR ORF; c.-456_-453del CCTT		Germline; No LOH	139
5′-UTR ORF; c.-80C > T		Germline: No LOH	140
5′-UTR ORF; c.-32_-29del		Germline; ND LOH	141
5′-UTR ORF; c.-29_-26delAGAGz		Germline; LOH	142
5′-UTR (−7) ORF; ATG-7G > C		Germline; No LOH	143
nt59_77dup19	fs, K25fs	Germline; LOH	144
c.163G > A	ms, p.A55T	Germline; ND LOH	145
c. 206C > T	ms, p.P69L	Germline; ND LOH	146
c.227G > A;	ns, W76*	Germline; No LOH	147
c.283C > TC	ms, p.P95S	Germline; ND LOH	143
c.371delCT	fs, 145*	Germline; No LOH	148
c.374_375delCT	S125*	Germline; No LOH	149
595T > C stop codon	Stop > Q	Germline; ND LOH	143
Sporadic Parathyroid Adenomas or Primary Hyperparathyroidisms			
c.25G > A	ms, p.G9R	Germline; No LOH	150
c.397C > A	ms, p.P133T	1. Germline; no LOH 2. ND; no LOH	150
c.582del259	Multiple abnormalities	Somatic; LOH	150
c.378G > C	ms, p.E126D	Germline; No LOH	151
Hairy Cell Leukemia			
c.500delC	fs, A167Qfs	Somatic	152
c.180G > A	W60*	Somatic	152
c.283G > C	ms, p.E80Q	Somatic	152
c.333-353del21	G111del6	Somatic	152
C475+1G > A	Splice acceptor variant	Somatic	152
C475+1G > T	Splice acceptor variant	Somatic	152
c.596A > C	X199S	Somatic	152
c.281C > T	ms, p.P94L	Somatic	152
c.5C > G	S2*	Somatic	152
c.58C > T	Q20*	Somatic	152
c.87C > A	C29*	Somatic	152
c.179G > A	W60*	Somatic	152
c.227G > A	W76*	Somatic	152
Breast Cancer			
c.80_83delCGGC	fs, C29fs	Somatic	153
c.285_286insC	fs, K96fs	Somatic	153
c.349C > T	ms, p.P117S	Somatic	153
c.408_475+7del75	p.?	Somatic	153
Small Intestine Neuroendocrine Tumors			
c.110-122del13	fs, D37fs	Somatic	154
c.174-177del4	fs, R58fs	Somatic	154
c.195delG	fs, Q65fs	Somatic	154
c.280insC	fs, P94fs	Somatic	154
c.280delC	fs, P94fs	Somatic	154
c.285-286insA	fs, P95fs	Somatic	154
c.353-354ins AA	fs, K118fs	Somatic	154
c.372-374delCT	fs, K118fs	Somatic	154
c.386insT	fs, H129fs	Somatic	154
c.408-412del5	fs, D136fs	Somatic	154
c.528delC	fs, D176fs	Somatic	154
c.560-563del4	fs, T187fs	Somatic	154
c.573-574delTG	fs, P191fs	Somatic	154
c.591-594del4	fs, Q197fs	Somatic	154
c.278_299 del	fs. Arg93fs	NA	155
c.551delT	fs. V184fs	Somatic	155
c.418delA	fs. S140fs	NA	155
c.160G > T	fs. G54*	Somatic	155
c.34_49del	fs. S12fs	NA	155
c.90_104del	fs. Arg30_P35 delinsArg	NA	155
c.390_391delGG; c.939_398del	fs. L130fs	NA	155
c.118G > T	fs. G40*	Somatic	155
c.414_423del	fs.s138fs	Somatic	155
c.202A > T	fs. L68*	Somatic	155
c.280delC	fs. P94fs	NA	155
c.529delG	fs. G177fs	Somatic	155
c.275_276insT	fs. P92fs	NA	155
c.374_375insT	fs. S125fs	Somatic	155
c.279_280insC	fs. P94fs	Somatic	155
c.596A > T	fs.*199L s	Somatic	155
c.229C > T	fs. G77fs	NA	155
c.127delCinsTAA	fs.R43fs	Somatic	156
c.279_280insT	fs.P94fs	Somatic	156
c.334delA	fs.S112fs	Somatic	156

Table 1 (*continued*)

Mutation	Protein change	*CDKN1B* status	Reference
c.518A > G	ms.N173S	Somatic	156
c.125C > T	ms.T42I	Somatic	156
AIP mutation-negative familial isolated pituitary adenoma			
c.286A > C	p.K96Q	Germline	157
c.356C > T	p.I119T	Germline	157
Familial colorectal cancer			
c.195G > T	ms, p.Q65H	Germline	158
T-cell prolymphocytic leukemia			
c.118G > T	ms, p.E40X	Somatic	159
Gigantism and neurodevelopmental defects			
5′-UTR ORF; c.-73G > A		Germline	160

NA, Not available.

model showing a tumor spectrum overlapping human MEN1/MEN2) presented a causative *germline CDKN1B* homozygous frameshift [147]. In the same paper, the authors reported a case of human MEN (negative for *MEN1* alterations) showing a *CDKN1B germline* nonsense mutation (TGG- > TAG mutation at codon 76) [147]. After this pivotal study, a significant number of investigations demonstrated the occurrence of *germline CDKN1B* changes in MEN1-like cases, in which *MEN1* gene alterations were not evidenced [139–149,163,164]. These cases were therefore classified as MEN4 [164]. Genetical details of the MEN4 cases reported so far are listed in Table 1 [139–149]. In some circumstances LOH was demonstrated in the tumor specimens, while in others only one allele was altered, pointing out the complexity of the underlining genetic mechanisms.

Hairy cell leukemia (HCL)

HCL is a form of B-cell CLL (Chronic Lymphocytic Leukemia) in which the B lymphocytes develop cytosolic projections similar to hair-like microvilli. The disease, characterized by a progressive pancytopenia, infiltrations of bone marrow and hepato- and splenomegaly, is generally due to *BRAF* mutation, mostly BRAFV600E [165]. However, in some HCL variants *BRAF* mutations could not be detected. Recently, investigating some HCL cases by employing whole-exome sequencing and targeted DNA sequencing analysis, *CDKN1B* has been identified as the second most frequently altered gene (16%) in this type of lymphoma. The mutations are clonal (not occurring in the germ-line), in the majority of cases are in heterozygosity and are frame-shifts [152,166].

Small intestine neuroendocrine cancer

The incidence of small intestine neuroendocrine tumors (SI-NET) is progressively increasing and, differently from other NET forms, the underlying genomic alterations have not been identified yet. Recently, by means of SI-NET exome- and genome-sequencing analyses, recurrent somatic mutations and deletions of *CDKN1B* have been detected. In particular, *CDKN1B* frameshift mutations have been identified in 14 out of 180 cases. Furthermore, hemizygous deletions embracing *CDKN1B*, have been identified in 7 out of 50 patients, pointing to *CDKN1B* alterations as driver mutations in SI-NET [154–156].

Breast cancer

All cancers, including breast cancers, show several somatic genomic changes. However, only a limited number of these mutations give a selective advantage and might be considered as driver mutations and not passenger changes. In 2012, an extensive analysis of coding exons of 21,416 genes in 100 breast cancers identifies novel driver genes, including *CDKN1B* [153]. The changes did not occur in *germlines*, but were acquired during the transformation process (See Table 1) [153].

Other cancers

Several other human tumors were reported to show *CDKN1B* mutations (including missense and frameshift changes) or deletions in chromosomal regions embracing *CDKN1B* encoding gene. These cancers include solid tumors (prostate cancer, familial isolated pituitary adenomas, sporadic parathyroid adenomas, pheochromocytomas) and hematological cancers (mieloproliferative disease, acute myeloid leukemias and adult acute myeloid leukemia) [167–169]. Additional details are reported in Table 1. Although not definitely established for all the genetic changes, haploinsufficiency is the most probable consequence of all these alterations. In this context, it is also to underline that the control of CDK activity (particularly CDK2) might be important not only for the malignant transformation process but also for creating an environment favouring cancer growth (like neoangiogenesis) [170].

Final considerations

Compelling evidence indicate that p27 is a tumor suppressor protein. Considering the overall available findings and disregarding the data reported in only few instances, a general conclusion is that an insufficient CKI amount or a p27 mislocalization (i.e. abnormally high cytosolic *versus* nuclear content) might favor malignant transformation, development of an aggressive phenotype and anticancer therapy resistance. Accordingly, based on its role in cytosol and nucleus, p27 might be represented as *Janus*, a roman god usually depicted with two faces. The numerous experimental data suggest that a threshold is necessary for allowing the CKI to act as a tumor suppressor protein. However, other aspects of the interplay p27/cancer need to be taken into considerations. First of all, while the quantitative reduction of p27 KID region might be balanced by the corresponding $p21^{Cip1}$ or $p57^{Kip2}$ KIDs, changes of p27 C-terminus levels/localization could affect more significantly malignant phenotype. As a matter of facts, p27 C-end has several interactions, perhaps not all characterized in details. The majority of the existing findings point to the modulation of cytoskeleton and cell movement as major activities of p27 C-end. The motility and invasion are key aspects of cancerogenesis, strictly interconnected to the metastatization process. Thus, future studies should shed light on this still unclarified p27 function. A second feature that needs to be unravelled is the interplay between the microenvironment in which malignant cells (including cancer stem cells) live and p27 level, cellular localization and metabolism. Particularly, the low level of oxygen and the occurrence/absence of specific nutrients is now well known to affect significantly the components of

the cell cycle engine, including one of its major actors as p27. This represents, in our view, a further key scenario.

Moreover, the consequence of p27 mutations (including missense or nonsense changes) must be evaluated not only, as generally reported, on the basis of their effects on protein amounts. For example, the expression of a short or altered p27 might affect the activity of the normally produced protein (resulting from the normal allele transcription). In other words, altered p27 might have a dominant pro-oncogenic effect. Such mechanism, to the best of our knowledge, has not been investigated. An alternative hypothesis is that mutations might affect by various mechanisms the p27 phosphorylation pattern, shifting it from a tumor suppressor protein into a dominant oncogenic protein. Furthermore, change in not directly phosphorylatable aminoacids might modify the pattern of phosphorylation by introducing or abrogating *consensus* sequences for specific kinase.

Finally, considering the frequency of *CDKN1B* mutations and/or, more in general, its down-regulation in endocrine tumors, a critical aspect to unravel is the role that p27 plays in the physiology of these specific tissues [171].

In conclusion, p27 is still a strong enigmatic protein and it is an easy prediction the unravelling of its further important and, possibly, unexpected roles in human cancerogenesis, metastatization, cancer cell staminality and response to treatments.

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Conflict of interest

None declared.

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