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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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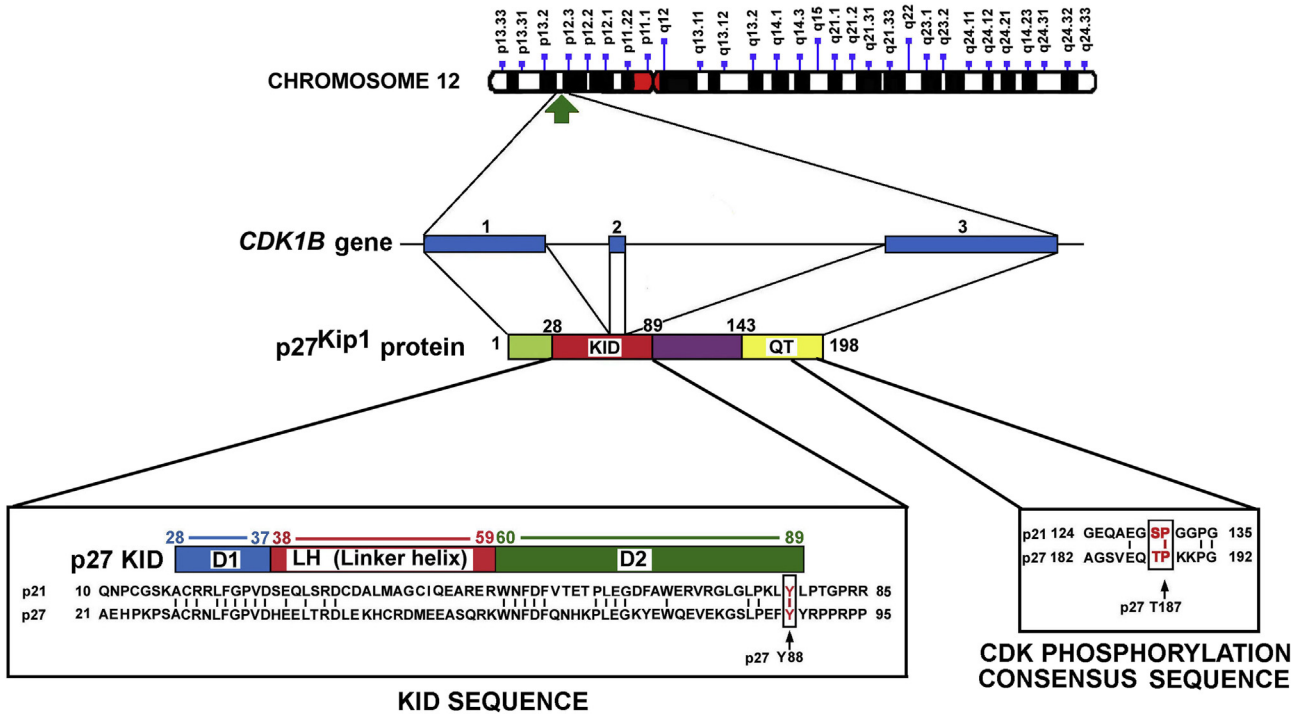

Fig. 1. Genomic *CDKN1B* organization and p27^{Kip1} protein domain structure. The figure reports (from the top to the bottom), the genomic localization of *CDKN1B* (chromosome 12p13), the exon-intron organization of the gene and the domain structure of p27^{Kip1}. Two sequences of the human p27^{Kip1} (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].