Introduction

The transition between cell cycle phases of eukaryotic cells is controlled by a set of cyclin/cyclin-dependent kinase (CDK) complexes whose activity is in part regulated by the level, localization, and phosphorylation status of both protein partners. Additionally, interactions with specific proteins, defined as CDK inhibitors (CKI) or CDK regulators (CKR) may modulate the cyclin/CDK complex function. On the basis of their sequence homology and specificity of action, CKRs are divided into 2 distinct families: INK4 and Cip/Kip. Members of the INK4 family (i.e., p15Ink4b, p16Ink4a, p18Ink4c, and p19Ink4d) specifically inhibit the activity of CDK4 and CDK6, whereas Cip/Kip members (i.e., p21Cip1, p27Kip1, and p57Kip2) control a broader spectrum of cyclin-CDK complexes.

Until recently, Cip/Kip family members were viewed as proteins with the primary function of modulating cyclin-CDK activity and, hence, cell proliferation. Currently, sound evidence indicates that the functions of Cip/Kip proteins are well beyond those of a simple CDK regulator. Accordingly, the activity of a specific Cip/Kip protein is only partially surrogated by the other family members.

Here we review the published data on p57Kip2 protein, the least-studied Cip/Kip member. We focus our attention in particular on the importance of p57Kip2 downregulation in human cancers and its relevance in tumor-targeted therapy.

Structure and Regulation of the CDKN1C Gene

In humans, the gene encoding p57Kip2 (properly called the CDKN1C gene) is localized at the 11p15.5 locus and encompasses 4 exons divided by 3 introns (1). The gene is remarkably rich in CpG islands situated both upstream and downstream from the putative transcriptional start site, resulting in a strong epigenetic control of its expression (2). As illustrated in Fig. 1, the human 11p15.5 locus harbors several imprinted genes that show a clear parent-of-origin specific expression. Of interest, the mouse homolog region (the distal region of chromosome 7) presents an identical cluster of linked genes, suggesting either the importance of their coordinate regulation or the occurrence of conserved regulatory mechanisms.

The control of 11p15.5-imprinted genes is achieved by 2 distinct imprinted control regions (ICR): ICR1 (or H19-ICR) and ICR2 (or KCNQ1/KvDMR-ICR). ICR1
is telomeric to 11p15.5 and modulates the imprinting of \( H19 \) and insulin-like growth factor 2 (\( IGF2 \)) by restricting access to enhancers [i.e., ICR1 acts as a chromatin insulator (3)]. As a result of this mechanism, \( IGF2 \) is paternally expressed, whereas \( H19 \) is maternally transcribed. ICR2 regulates the imprinting status of a region (the centromeric area of 11p15.5) that includes \( KCNQ1 \) (\( KvLQT1 \) or potassium voltage-gated channel, KQT-like subfamily member 1), \( KCNQ1OT1 \) (also known as \( LIT1 \), \( KCNQ1 \)-overlapping transcript 1, or long QT intronic transcript 1), \( PHLDA2 \), \( SLC22A18 \), and \( CDKN1C \) (Fig. 1; refs. 3–8). In particular, \( CDKN1C \) is maternally expressed, although a faint paternal allele expression is observed in most human tissues (9).

The results of a computer-aided analysis in 2001 suggested that numerous consensus sequences for different transcription factors occur in the \( CDKN1C \) promoter (10); however, only some of these sequences have been experimentally confirmed.

Several stimulatory protein-1 (Sp1) responsive elements are present in the \( CDKN1C \) promoter (10). Detailed studies have identified the region of interaction with Sp1 [from −87 to −113 bp from the transcriptional starting point (11, 12)]. Of importance, the binding of Sp1 to \( CDKN1C \) promoter is remarkably enhanced by histone hyperacetylation, reinforcing the importance of epigenetic mechanisms in the regulation of \( CDKN1C \) expression (Fig. 1; refs. 11 and 12).

In the context of epigenetic control, the proteins SMARCB1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1) and CITP2 (chicken ovalbumin upstream promoter transcription factor interacting protein 2) have been reported to increase (SMARCB1) or decrease (CITP2) \( CDKN1C \) expression by chromatin structure remodeling (13, 14). Intriguingly, SMARCB1 has been reported to be absent (mostly by gene deletion) in rhabdoid tumor, an aggressive pediatric malignancy that affects the kidney and central nervous system. SMARCB1 is part of an ATP-dependent, multiprotein, SWI/SNF chromatin remodeling complex, and it plays a role in controlling histone H3 and 

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**Figure 1.** Structure of the 11p15.5 region, genes located in the 11p15.5 area, and the \( CDKN1C \) gene. White boxes and gray boxes of paternal and maternal alleles represent expressed and nontranscribed genes, respectively. The arrows show the direction (toward the centromere or telomere) of transcription. The direction of TRPM5 gene expression is not known. The structure of the \( CDKN1C \) gene is also shown. In this case, white regions of boxes are UTRs, and gray regions form the coding sequence. Part of the \( CDKN1C \) reporter sequence is shown, including the TATA box and the sequences that interact with Sp1 and CTIP2 transcription factors.
H4 acetylation. Because of its activity in the regulation of chromatin structure, SMARCB1 induces CDKNIC gene expression. In turn, the absence of SMARCB1 in muscle cells results in reduced p57Kip2, increased cell proliferation, and downregulation of cell differentiation (14).

Epigenetic mechanisms have also been suggested to explain CDKNIC regulation by histone methyltransf erase EZH2. Polycomb protein Enhancer of Zeste 2 (EZH2) is frequently overexpressed in human cancers and has been associated with cancer aggressiveness. EZH2 specifically methylates lysine 27 of histone H3, a repressive chromatin mark associated with gene silencing. EZH2-mediated gene silencing requires histone deacetylase (HDAC) activity, and its functional relationship with DNA methylation is also of current interest (15).

A putative erythroblastosis virus E26 oncogene (ETS) consensus sequence has been identified in the CDKNIC promoter, but detailed studies suggest that only the related EWS-FLI-1 transcription factor modulates CDKNIC transcription, probably by an indirect inhibitory mechanism (10).

A positive modulator of CDKNIC transcription is Early Growth Response 1 (EGR1). This observation was initially reported by Svaren and colleagues (16) and subsequently confirmed by others (11, 17). The importance of this finding is related to the rapid and strong EGR1 increase induced by growth factors, mitogens, cytokines, environmental and mechanical stresses, and DNA damage. However, the ability of EGR1 to activate CDKNIC expression is reduced by EGR1 binding to PAX3-FOXO1. Chimeric protein PAX3-FOXO1 is encoded by a gene that originates from the translocation between chromosomes 2 and 13. Thus, the oncogenic effect of the chimeric PAX3-FOXO1 protein seems to be due, at least in part, to the inhibitory activity on EGR1, the reduced CDKNIC expression, and the absence of complete myogenic differentiation.

A glucocorticoid response element has also been identified in CDKNIC gene promoter, although its importance has not been clarified yet. Of note, however, in several experimental models, it was shown that dexamethasone specifically regulates CDKNIC transcription and p57Kip2 levels (18). Moreover, a functional glucocorticoid response element has also been observed in the mouse CDKNIC promoter. Thus, the induction of p57Kip2 upon glucocorticoid treatment may be putatively considered one of the molecular mechanisms responsible for the antiproliferative effects of these steroids.

A complex and undefined interplay links CDKNIC activity with p73 and p63 transcription factors. It has been reported that the p73 isoform upregulates the expression of both p57Kip2 and KCNQ1. The importance of this observation remains to be established (19). Because CDKNIC-negative mice share a similar phenotype with p63 knockout mice, Beretta and colleagues (20) examined the possibility of a functional link between the 2 proteins. They showed that a p63 isoform (ΔNp63α) transactivates CDKNIC by binding to its promoter in 3 different sequences (20). Although this interplay has not been definitely shown, it may be relevant for the development of ectodermal tissues, including limb, skin, and, in general, multilayered epithelia. Indeed, the ΔNp63α isoform is thought to be a master gene in the asymmetric division of epithelial cells. It is also involved in the pathogenesis of several human diseases phenotypically characterized by ectodermal dysplasia. Thus, the possibility that the p63 isoform might regulate p57Kip2 levels is garnering considerable interest that guarantees further investigation.

Hairy and enhancer of split (Hes1), a transcription factor whose expression is initiated by the Notch signaling pathway, is a repressor of cell cycle inhibitors. It has been shown that in early pituitary development, at embryonic day (E) 10.5, cells contained in the Rathke’s pouch of Hes1 mutant mice have decreased proliferation, indicated by changes in histone H3 phosphorylation (21). Furthermore, pituitary glands lacking Hes1 exhibit decreased cell proliferation, shown by significant upregulation of the cyclin-dependent kinase inhibitors p27Kip1 and p57Kip2, from E 10.5 to E 14.5 (21). Consistent with its unique role in embryogenesis, p57Kip2, but not p21Cip1 or p27Kip1, is induced by the transcription factor E47, a protein that is essential for human development. Of interest, inhibitor of differentiation 2 (Id2), a natural inhibitor of E47, suppresses p57Kip2.

PLAG1/ZAC1 is an imprinted gene that is selectively expressed by the paternal allele from chromosomal region 6q24. It has been shown that ZAC1 affects the expression of p57Kip2 (22).

The identification of microRNAs (miRNA or miR) has added a new layer of complexity to the regulation of gene expression. miRs are noncoding single-strand RNAs, ~22 nt long in their mature forms. miRs bind to the 3′ untranslated region (3′-UTR) of target mRNAs through base pairing, resulting in mRNA cleavage or, most frequently, translation inhibition. One miR might regulate multiple gene transcripts because it can bind to its targets with both a perfect and imperfect complementarity. Therefore, given that the estimated number of human miRNAs is ~1,000, miRs should control >30% of human genes. Accordingly, miRs might be thought of as key regulators of normal and pathological phenotypes, including cancer and cardiovascular disease.

Recently, 2 miRs (miR-221 and miR-222) were reported to downregulate the expression of both p27Kip1 and p57Kip2 transcripts (23–28). Intriguingly, miR-221 and miR-222 also control the transcripts of several other important genes, including Kit, Gas6/Mxoa2, Pten, Timp3, Bmf, Mdm2, Stat, and Puma. Changes in the levels of these miRs have been reported to be involved in the pathogenesis of cancer. Very recently, a significant breakthrough regarding the miR-221/222 mechanism of action was reported (29). It was shown that these miRs might be functionally underactive as a result of target site hindrance. Their activity is regained by the activation of a protein named Pumilio-1 (PUM-1). By interacting with specific 3′-UTR binding
sites, PUM-1 may induce a local change in RNA structure that favors association of miR-221 and miR-222 with their targets. Further studies are necessary to decipher the extent to which this interaction contributes to the control of miR-221/222 target levels.

In gastric cancer, CDKN1C expression is regulated by miR-25 (30), whereas in human embryonic stem cells it is specifically targeted by miR-92b (31). Functional analyses have shown that miR-dependent Cip/Kip silencing results in a shift in the number of cells from the G1 phase to the S phase. In murine embryonic stem cells and neural stem cells derived from embryonic stem cells, miR-106b and miR-17-92 suppress p57Kip2 and p21Cip1, promoting the process of self-renewal of these cells (32).

### Figure 2

Domain structure of p57Kip2 protein. Bottom, the CDK inhibitory domain (KID) includes 3 major sequences: the cyclin-binding region, the CDK-binding region, and the $3_{30}$ helix. The KID region of human p57Kip2 (hp57) is also compared with the KID of human p27Kip1 (hp27). Asterisks identify the conserved residues. The proteins that putatively interact with the hp57 KID domain (i.e., B-Myb, MusD, Mash1, Neuro D, and Nex/Mash) are also shown. Left, the sequence of the PAPA domain (from residue 142 to residue 219). The PAPA domain putatively interacts with the LIMK-1 protein. Top, the sequence of the C-terminal domain of human p57Kip2 protein. This domain (also known as the QT domain) shows similarity with the QT domain of hp27 (represented as boxes). Part of the QT domain also presents homology with the PCNA-binding domain of human p21Cip1 (hp21). In this case, asterisks identify the conserved residues in the PCNA-binding region between hp57 and hp21. The interaction of JNK/SAPK with the hp57 QT domain is also shown.

**p57Kip2 Protein**

**Structure of p57Kip2 protein and its interactors**

Human CDKN1C encodes a 316-amino acid protein that migrates at 57 kDa by SDS-PAGE electrophoresis. At the primary sequence level, p57Kip2 shows a modular structure organized into 3 distinct domains: (i) domain I (located at the N-terminal region), which comprises a CDK inhibitory domain (KID) that shares significant homology with the respective KIDs of p21$^{Cip1}$ and p27$^{Kip1}$; (ii) domain II, which contains multiple repeats of proline and alanine (i.e., the PAPA region); and (iii) domain III (located in the C-terminal region), which encompasses a conserved motif called the QT domain (Fig. 2).
The KID domain is necessary and sufficient to bind and inhibit CDK activity. It embraces 3 regions: a cyclin-binding sequence, a CDK-binding site, and a 310 helix (Fig. 2). The 310 helix contains an amino acid pair (phenylalanine-tyrosine) that mimics the adenine base of ATP and interacts inside the catalytic CDK2 cleft with the N-terminal end of the kinase (33). Thus, the 310 helix appears to be indispensable for the in vivo inhibition of cyclin A-CDK2 and cyclin E-CDK2 activities. The p57Kip2 amino-terminal domain (in contrast to p21Cip1 and p27Kip1) interacts with the transcription factor B-Myb (33), B-Myb competes with cyclin A2 for binding to p57Kip2, resulting in the release of active cyclin/Cdk2 kinase. Accordingly, in Saos-2 cells, B-Myb–forced expression partially prevents the G1 cell cycle arrest due to p57Kip2 (34).

The N-terminal domain of p57Kip2 is also the site of interaction with MyoD and other basic helix-loop-helix (b-HLH) transcription factors, such as Mash1, NeuroD, and Nex/Math2 (35–39). p57Kip2 has also been reported to interact with Nuclear Receptor Related 1 (NURR1) protein. However, this interaction was shown in cell models overexpressing both proteins. The site of binding has not been precisely mapped, although a p57Kip2 mutant that is unable to inhibit CDK activity is still capable of interacting and cooperating with NURR1 in its role of transcription modulation in the maturation of dopamine neuronal cells (40).

Whereas the p57Kip2 amino- and carboxy-terminal domains are conserved in mammals, the internal domain (domain II) of human p57Kip2 has been substituted in mouse with a proline-rich region (the proline domain) followed by an acidic repeat in which glutamic or aspartic acid occur every 4 amino acids. The p57Kip2 internal domain may be fundamental for functions other than CDK inhibition. This region of the mouse CKI was reported to interact with the N-terminal end of LIM domain kinase 1 (LIMK-1), a kinase involved in the control of actin dynamics and cytoskeletal organization. The interaction of p57Kip2 with LIMK-1 has also been confirmed in human cells (41–43).

The C-terminal region of p57Kip2 is homologous, in the QT box, to the corresponding p27Kip1 domain. It also shows similarities with p21Cip1 in that it presents a proliferating cell nuclear antigen (PCNA, a DNA polymerase processivity factor) binding domain [aa 271–275 (46)]. This allows p57Kip2 to interact with PCNA, although with a much lower affinity compared with p21Cip1, and in turn block processive DNA synthesis (46). In addition, the p57Kip2 QT domain interacts with and inhibits the kinase activity of c-Jun NH2-terminal kinase/stress-activated protein kinase [JNK/SAPK (47, 48)]. Of interest, p21Cip1 is also able to bind and suppress JNK/SAPK activity via its amino-terminal domain. Conversely, p27Kip1, which contains a QT domain, does not inhibit JNK/SAPK activity. Finally, but of importance for explaining the p57Kip2 localization, a putative nuclear localization signal might be recognized in the QT box domain of mouse and human p57Kip2 (49).

p27Kip1 and p57Kip2 are structurally related proteins that share a conserved KID sequence (Fig. 3) and a QT domain (shown in Fig. 2) in their NH2- and COOH-terminal regions. However, the internal domain (domain II) of p57Kip2 is totally absent in p27Kip1 (Fig. 3), suggesting a putative specific function of this sequence.

The amino acid composition of full-length p57Kip2 shows deviations from the average composition of globular proteins that are consistent with a protein exhibiting an intrinsic disorder. Hydrodynamic and spectroscopic analyses indicate that human p57Kip2 lacks a stable helical and β-sheet structure, although the formation of a secondary structure cannot be precluded (50). On the basis of these findings, p57Kip2 joins the most-investigated p27Kip1 and its sibling p21Cip1 in being considered highly unfolded molecules (51–54).

The Cip/Kip proteins are intrinsically unstructured and adopt specific tertiary conformations only after binding to their interactors. This conformational flexibility suggests
p57Kip2 metabolism

Overall, the cellular metabolism of p57Kip2 has been scarcely investigated. Few data are available regarding the shuttling of the protein from the cytosol to the nucleus and vice versa. However, a consensus sequence for nuclear localization (KRKR) may be putatively identified in the protein sequence. Information about the postsynthetic modifications of the protein is also scarce. A phosphorylation on threonine 310 has been suggested to be important for human protein degradation (56). The phosphorylation of serine 268 was also described in a study devoted to an extensive analysis of nuclear protein phosphorylation (57). In addition, the occurrence of p57Kip2 phosphorylated in tyrosine has been described (58). We recently showed, by bidimensional electrophoretic analysis, at least 6 p57Kip2 isoforms in K562 cells [an erythroleukemic cell line (Fig. 4; ref. 59)]. Treatment with protein phosphatase showed that phosphorylation events and protein-protein interactions might be fundamental in driving the folding of the CKIs, thereby modulating their ability to inhibit cyclin-CDK complexes (55). Likewise, it may explain why Cip/Kip family members have a broad specificity for CDK proteins and are capable of interacting with a wide diversity of proteins to regulate various cellular functions (55).

p57Kip2 roles

p57Kip2 and cell cycle regulation. p57Kip2 protein was initially characterized as an inhibitor of all cyclin-CDK complexes, albeit displaying lower affinity toward cyclin B-CDK1 and cyclin D2-CDK6. Similar to p21Cip1 and p27Kip1, p57Kip2 was also reported to enhance binding between cyclin and CDKs, thus forming an active complex (62). However, although the interactions of p21Cip1 and p27Kip1 with the cyclin-CDK complexes have been extensively examined, no further characterization has been performed in the case of p57Kip2.

Consistent with its role as a CDK inhibitor, p57Kip2 expression levels are high in the G0 and G1 phases of the cell cycle and decrease during progression from the G1 to S phase simultaneously with the activation of cyclin-CDK complexes (63, 64). Accordingly, overexpression of p57Kip2, like that of p27Kip1, induces G1 arrest in cultured cells. p57Kip2 has also been correlated with endoreduplication. Endoreduplication is replication of the nuclear genome in the absence of cell division, which leads to elevated nuclear genome content and polyploidy. p57Kip2 protein levels oscillate periodically during subsequent endoreduplication cycles of trophoblast giant cells, diminishing before S entry and increasing after S phase completion (65). The role of p57Kip2 in endoreduplication is supported by the observation that p57Kip2 suppresses CDK1 activity in mouse trophoblast stem cells, thus preventing cytokinesis.

p57Kip2 controls organogenesis, embryogenesis, and cell differentiation. The expression profile of p57Kip2 is characterized by a high temporal, spatial, and cellular specificity that is evident not only during fetal development but also in postnatal and adult life. During embryogenesis, p57Kip2 is found in derivatives of all 3 germ layers (the ectoderm, mesoderm, and endoderm) and in all major organs of the developing fetus. In several organs, p57Kip2 protein peaks at role in the homeostasis of p57Kip2 protein levels (60, 61). p57Kip2 ubiquitination involves, as E3 ubiquitin-ligase, the Skp, Cullin, F-box-containing complex (SCF complex), which usually modifies target proteins from the late G1 to early M phase. SCF complex consists of 3 constant subunits (SKP1, CUL1, and RBX1) plus a variable F-box protein that recognizes and interacts with the target protein. The first identified F-box protein involved in p57Kip2 ubiquitination was the S-phase kinase–associated protein 2 (SKP2), which also mediates p27Kip1 and p21Cip1 recognition. As for p27Kip1, SKP2 interacts with p57Kip2 and promotes its degradation in a phosphorylation-dependent manner (60). The p57Kip2–recognized phosphodegron is phosphothreonine 310 (corresponding to phosphothreonine 329 in mice). An SKP2-independent mechanism of p57Kip2 ubiquitination in TGFβ1-mediated inhibition of osteoblast cell differentiation has also been reported (61). The cytokine upregulates, via the SMAD pathway, the expression of FBL12, a different F-box protein expressed in the limb bud of developing embryos. FBL12 substitutes for SKP2 in the E3 complex (the SCF-FBL12 complex) that is responsible for p57Kip2 degradation.

![Phospho-p57 Isoforms](image)

**Figure 4.** p57Kip2 protein isoforms. A sample of K562 cell extracts (500 µg protein) was initially separated by bidimensional electrophoresis. The gel was then blotted on nitrocellulose and analyzed with the use of rabbit anti p57Kip2 antibodies. At least 6 spots were observed corresponding to the following isoelectric points: isoform 1 (PI 5.4), isoform 2 (PI 5.28), isoform 3 (PI 5.25), isoform 4 (PI 5.15), isoform 5 (PI 5.0), and isoform 6 (PI 4.9). Isoform 1 corresponds to the wild-type protein, and isoforms 3–6 are phosphorylated isoforms as revealed by protein phosphatase treatment (59). Isoform 2 has not been characterized yet.
key differentiation stages and declines to low or undetectable levels thereafter.

The value of p57Kip2 in embryogenesis is highlighted by the finding that CDKN1C-null mice have high neonatal mortality and severe developmental defects. The knockout mice exhibit gastrointestinal tract defects, omphalocele, limb shortening due to abnormal endochondral ossification, cleft palate, adrenal cortex enlargement, renal medullary dysplasia, lens-fiber cell abnormalities, and increased body weight (66–68). Of interest, p57Kip2 excess in mice increases embryonic lethality and decreases body size, suggesting that embryonic growth requires an accurate control of p57Kip2 dosage (66–68). CDKN1C-null mice also show several placental abnormalities, including trophoblastic dysplasia.

Unlike the other 2 members of the Cip/Kip family (p21Cip1 and p27Kip1), p57Kip2 has a tissue-restricted expression pattern during both adult and embryonic life. The p57Kip2 transcript may be evidenced in skeletal muscle, brain, heart, lung, kidney, pancreas, testis, and placenta. p57Kip2 participates in lens-fiber differentiation (69, 70) and maturation of the nervous system (71). The protein regulates neuronal migration in mouse cortex and rat neurogenesis (72, 73). p57Kip2 downregulation facilitates oligodendrocyte differentiation (44, 74, 75) and is required for dopamine neuron maturation (40) and mouse retina development (76). Finally, p57Kip2 downregulation regulates in vitro myelination by Schwann cells (77).

A large number of independent studies also suggest that p57Kip2 actively participates in myogenesis (78). In particular, CKI is able to stabilize MyoD, a transcription factor that is highly specific for muscle involved in myogenic differentiation. p57Kip2 is also involved in the differentiation of several other cell phenotypes, including podocytes (79), placental cells (65, 80, 81), keratinocytes (82), pancreatic cells (83), hepatocytes (84, 85), T-lymphocytes (86), spermatozoa (87, 88), Leydig cells (88), chondrocytes (89–91), and adrenal cortex cells (92).

In particular, podocytes’ terminal differentiation is characterized by an increase of p57Kip2 associated with specific variations in cyclin D levels (79). Moreover, coordinated changes of p57Kip2, p27Kip1, and p21Cip1 have been shown clearly during keratinocyte maturation (76) and human placenta development (65, 80, 81).

Although CDKN1B and CDKN1C knockout mouse have distinct phenotypes, a knockin mouse model in which CDKN1C was replaced with CDKN1B showed a significant correction of most CDKN1C developmental defects (93). This finding supports the notion that differences between CDKN1B and CDKN1C knockout mice are due mostly to changes in the temporal and spatial expression of p27Kip1 and p57Kip2 proteins, and only in part to differences in their intrinsic molecular activities (93). However, some defects of CDKN1C knockout mice are not relieved in p27Kip1−/−p57Kip2−/− knockin mice. These include abnormalities of the renal papilla, placenta, and abdominal wall (93). It is not yet clear whether the lack of correction is due to different molecular activities of the 2 proteins or to differences in postsynthetic metabolism that can affect their relative cellular levels.

**Role of p57Kip2 in hematopoiesis.** Hematopoietic progenitors simultaneously perform programs of differentiation and proliferation. One well-established linkage between the 2 programs occurs during terminal differentiation, when cell cycle exit is brought about by the induction of CDK inhibitors. However, it is unclear whether coordination between cell growth and differentiation occurs prior to cell cycle exit.

A number of reports have correlated the process of hemopoiesis with the content of p57Kip2 in so-called hematopoietic stem cells (HSC). In 2004, Scandura and colleagues (94) showed that the addition of TGF1 to primary human cord blood–derived CD34+ hematopoietic progenitors caused an increased transcription of CDKN1C and accumulation of CKI. The authors concluded that they identified the molecular pathway by which TGF1 mediates its cytostatic effects on human hematopoietic cells (94). A subsequent investigation suggested that p57Kip2 is responsible for the G0/G1 block of HSC and that its decrease is required for S entry (95). Two recent articles (96, 97) confirmed the central role played by decreased p57Kip2 in the exit from quiescence and reentry into the cell cycle, which are essential for HSC self-renewal and regeneration. The first investigation showed that SKP2 expression (and thus p57Kip2 degradation) is increased in HSC and progenitors in response to hematopoietic stress from myelosuppression or after transplantation (96). The study also suggested a previously unrecognized role for SKP2 in regulating HSC and progenitor expansion and hematopoietic regeneration after stress. The other, more recent study (97) showed that elimination of the longer PR domain–containing isoform encoded by the Mecom locus (PR domain–containing ME) leads to a reduction in the number of HSCs and a complete loss of long-term repopulation capacity, while the HSC compartment is shifted from quiescence to active cycling. The authors identified in p57Kip2 the effects of the PR domain–containing ME regulatory activity on the long-term function of HSCs.

It was recently shown that a novel linkage exists between cell cycle progression and red blood cell commitment, and that it occurs several cell division cycles before the exit from the cell cycle (98).

This connection consists of suppression, rather than induction, of CDKN1C expression. In turn, a decrease in p57Kip2 causes the cells to enter the S phase rather than exit the cell cycle. In particular, it has been shown that the downregulation of p57Kip2 (and the S-phase entry) commits the cells to erythropoietic differentiation, which is associated with the onset of erythropoietin dependence, activation of GATA-1 (a key erythroid transcriptional regulator), and a switch of chromatin conformation at the β-globin locus toward an active status. These findings identify an important role of p57Kip2 as a master regulator of erythroid differentiation, distinct from its activity as a CDK inhibitor (98).

**Role of p57Kip2 in cytoskeletal organization.** The cytosolic localization of p57Kip2 has been shown in all cells.

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and tissues investigated (see the Human Protein Atlas at http://www.proteinatlas.org). Although the function of the cytosolic protein has not been investigated in detail, several lines of evidence suggest that at least one role is the regulation of the cytoskeletal dynamics and cell motility (41). Accordingly, the absence p57Kip2 causes a delayed migration of neurons in the cortical plate during mouse development (73).

In 2003, Yokoo and colleagues (41) reported that, in mouse osteoblastic cells, p57Kip2 binds to the actin cytoskeleton-modifying enzyme LIMK-1 and translocates it from the cytoplasm to the nucleus. Therefore, the nuclear sequestration by p57Kip2 reduces LIMK-1’s inhibitory activity on cofilin, the actin-binding protein that disassembles actin filaments. Once it is phosphorylated by LIMK-1, cofilin can no longer bind to actin, resulting in an increase of actin polymers and stress fiber formation. In accord with these data, it was shown that suppression of p57Kip2 by small hairpin RNA in Schwann cells induces cellular differentiation, even in the absence of axons (42). Of interest, p57Kip2 transfection in rat Schwann cells increases immunofluorescence staining for p57Kip2 and LIMK-1 in the nuclei, suggesting that p57Kip2 can bind to and actively translocate LIMK-1 (42).

Similar results were obtained on a cultured precursor of oligodendroglial cells (43). The levels of p57Kip2 mRNA and protein were evaluated in the course of an experimental autoimmune encephalomyelitis in rats. In particular, p57Kip2 expression is transiently downregulated under inflammatory, demyelinating conditions before the onset of disease remission, suggesting that p57Kip2 is an intrinsic inhibitor of oligodendroglial (re)differentiation. Accordingly, long-term silencing of p57Kip2 revealed that differentiation of cultured oligodendroglial cells is accelerated in response to lowered p57Kip2 levels.

In contrast to these data, Vlachos and Joseph (44) confirmed in a human cervical adenocarcinoma (HeLa) cell line the interaction between p57Kip2 and LIMK-1. However, they showed that this interaction does not result in the translocation of the kinase into the nucleus, but instead augments LIMK-1 activity, independently of its activator Rh0-associated kinase. In turn, LIMK-1 activation increases cofilin phosphorylation and hence actin-fiber formation.

p57Kip2 and apoptosis and senescence. In 2002, Samuelsson and colleagues (99) reported that p57Kip2 enhances staurosporine-induced apoptosis in HeLa cells in a CDK-inhibitory–independent manner. This finding was confirmed 5 years later by Vlachos and colleagues (100), who showed that selective p57Kip2 expression sensitizes cancer cells to apoptotic agents, such as cisplatin, etoposide, and staurosporine, via a mechanism that does not require p57Kip2–mediated inhibition of CDK but, rather, involves p57Kip2 translocation into mitochondria and activation of the intrinsic apoptotic pathway. In H1299 (a lung cancer cell line) and HCT116 (a colorectal carcinoma cell line), silencing of p57Kip2 was shown to suppress p73-mediated apoptosis induced by cisplatin treatment (101). Similarly, a p73 increase in postmitotic hNT neurons induced p57Kip2 and BAX, leading to apoptosis.

In some experimental models, p57Kip2 showed antiapoptotic activity, whereas other studies revealed no correlation between p57Kip2 and apoptosis (47, 102). Therefore, p57Kip2 relevance in apoptosis is probably confined to specific conditions and cell types.

In human astrocytoma cell lines, p57Kip2 accumulation reduced proliferation and induced a senescent phenotype (103). A similar p57Kip2–induced, senescence-like phenotype was observed in a PCC-1 prostate cancer cell line (104). However, despite these sporadic observations, the interplay between p57Kip2 and cellular senescence remains uncertain, in contrast to the well-established role of other CDK inhibitors.

p57Kip2 and transcriptional regulation. Like the other Cip/Kip proteins, p57Kip2 can repress transcription indirectly by inhibiting cyclin-CDK complexes, in turn preventing the phosphorylation of Rb-family proteins (p107, p110, and p130). The protein can also modulate the activity of transcription factors by binding them directly. As matter of fact, the N-terminal region of p57Kip2 can interact with MyoD and other b-HLH transcription factors, such as Mash1, NeuroD, and Nex/Math2 (39). p57Kip2 also modulates NURR1 activity in regulating gene expression in dopaminergic neuron development (40).

Ma and colleagues (105) recently showed that p57Kip2 causes a dramatic reduction of phosphorylation on serine 2 and serine 5 of the RNA polymerase II (pol II) C-terminal domain (CTD). The effect is due to p57Kip2 binding with E2F1 mediated by 2 E2F1 domains—one located in the central region of E2F1 and the other at the C-terminus. E2F1 then directs p57Kip2 to chromatin. Finally, p57Kip2 inhibits CKD7 and CKD9 (putative RNA pol II CTD kinases), affecting CTD phosphorylation and in turn RNA synthesis (105). The authors proposed that these results highlight a novel mechanism of tumor suppression by p57Kip2; however, further confirmation is needed to substantiate this promising conclusion.

p57Kip2 and Human Cancers

In a study by Takahashi and colleagues (106), p57Kip2–null mice showed clear tissue alterations but did not develop spontaneous tumors, at least during the 5-month observation period. This finding is similar to observations in p21Cip1/Cip1–/– mice, whereas p27Kip1+/– mice showed an increased risk of cancer development in only a few specific tissues. Thus, the data obtained in modified animals seem to rule out a relevant function of p57Kip2 in human carcinogenesis. However, the absence of malignancies in p57Kip2–null mice does not rule out a role for this protein in human tumors, because differences between mice and humans in the mechanisms of carcinogenesis hamper the possibility that data obtained in animals can be purely and simply extrapolated to the human clinical setting. Moreover, the
upregulation of proteins (i.e., the other members of Cip/Kip family) that might compensate for the absence of p57Kip2 in CDKN1C-null mice must be considered.

Experiments performed in several cellular models indicate that the immortalization process is frequently associated with a decrease in CDKN1C expression, and p57Kip2-forced overexpression reverses malignant phenotypes. However, these observations do not furnish direct proof of p57Kip2's role in malignant transformation or cancer development.

To assess the role of p57Kip2 in tumors, we now review the data available on CDKN1C status and p57Kip2 content in major human cancers.

**p57Kip2 content in human malignancies**

**Liver tumors.** Eight studies have been performed on p57Kip2 levels in liver cancers, including hepatocellular carcinomas (HCC) and hepatoblastomas. In 1998, Bonilla and colleagues (107) analyzed 17 HCC samples and did not find any CDKN1C mutations. A subsequent analysis performed on 15 HCC specimens showed an increased gain of imprinting at 11p15, associated with a loss of CDKN1C expression in ~30% of cases (108). Subsequently, more than 200 HCC samples were analyzed by immunohistochemistry in comparison with normal counterparts. These studies allowed the conclusion that p57Kip2 downregulation is frequently observed in HCC and represents an independent prognostic factor associated with a poor outcome.

Few cases of hepatoblastoma have been investigated. Surprisingly, in one such study the authors reported an increase of CDKN1C expression (109). Very recently, the expression of CDKN1C was studied in 44 HCC cases and compared with nm23 (a well-known metastasis suppressor) expression (110). The authors reported a protein decrease in ~50% of cases and showed that the abnormal coexpression of nm23/p57Kip2 may be a useful molecular marker for metastasis and unfavorable prognosis (110).

**Urothelial carcinomas.** Alterations of CDKN1C expression were investigated in bladder cancers at both mRNA and protein levels. Two studies performed on p57Kip2 transcript showed a decrease in 37% and 78% of cases, respectively (111, 112). However, in these studies only a total of 43 samples were analyzed. An immunohistochemical study performed on 31 patients affected by urothelial carcinomas of the bladder and 7 subjects with normal urinary bladder mucosa showed that the protein was absent in more than 70% of the cancer cases (23 out of 31; ref. 113).

**Pancratic carcinomas.** Possible alterations in CDKN1C gene status and its expression in pancreatic carcinomas were investigated in 2 studies (114, 115). Eighteen samples were analyzed for altered CDKN1C promoter methylation, and more than 50 samples were studied by immunohistochemistry for p57Kip2 protein content. The results showed that the protein was decreased in ~50% of cases and that the phenomenon correlated with the more aggressive forms.

**Colorectal cancers.** Two major investigations focused on p57Kip2 levels in colorectal carcinomas (116, 117). Both of these analyses employed an immunohistochemical technique. In a study analyzing 110 pairs of colorectal cancer and non-tumor tissues, the clinical and pathological survey showed a significant correlation between low expression of p57Kip2 and large size of the tumor (116). In 2003, Li and colleagues (117) used immunohistochemistry to detail changes in p57Kip2 during colorectal carcinogenesis. They examined 22 normal mucosa, 62 adenomas, 17 carcinomas in adenomas, 189 primary carcinomas, and 23 lymphonodes, and they reported a significant decrease during the transition from adenoma to carcinoma.

**Adrenocortical cancers.** p57Kip2 content was investigated in a series of 79 sporadic adrenal tumors (118). No CDKN1C mutations were detected, whereas a decreased expression, evaluated by reverse transcriptase PCR and immunohistochemistry, was observed. Subsequently, Bourcigaux and colleagues (119) investigated 31 adrenocortical tumors (11 benign and 20 malignant) and found the absence of p57Kip2 expression in 15 malignant samples. Intriguingly, the downregulation was shown to be associated with an increase of CDK2 activity.

**Thyroid cancers.** Three studies have been performed on the level of p57Kip2 in thyroid tumors (120–122). Ito and colleagues (120) investigated p57Kip2 content in different thyroid tumors. The protein was overexpressed in follicular adenomas and minimally invasive follicular adenomas. The upregulation diminished in invasive follicular carcinomas. p57Kip2 was reduced in poorly differentiated and undifferentiated carcinomas (120). The same group investigated p57Kip2 content in 49 cases of thyroid lymphoma and 10 cases of chronic thyroiditis, and they reported a significant reduction of p57Kip2 protein in more than 50% of thyroid lymphoma cases (121). In 2007, however, Melck and colleagues (122) did not observe p57Kip2 variation between 100 benign and 105 malignant thyroid lesions.

**Ovarian cancers.** In 2002, Sui and colleagues (123) examined 103 cases of epithelial ovarian cancer by immunohistochemistry and 26 by immunoblotting. They detected p57Kip2 in 21 out of 33 benign cancers, 12 out of 23 borderline cancers, and 19 out of 47 ovarian carcinomas. An analysis of 171 primary tumors from previously untreated patients with advanced ovarian carcinoma (stage III) showed a frequent decrease of the protein, without any correlation with poor survival (37, 124).

**Gastric cancers.** In a study by Shin and colleagues in 2000 (125), neither mutations of the CDKN1C gene (30 samples) nor its reduced expression in gastric cancer cell lines were reported. Conversely, p57Kip2 immunoblotting analysis in gastric cancer specimens showed lower levels of the protein (in 36 patients) compared with normal counterparts (126). The finding shows a good correlation with lymphonode involvement. Finally, 5 out of 35 (14%) gastric cancers showed CDKN1C gene hypermethylation (127).

**Oral and laryngeal cancers.** Fan and colleagues (128) investigated p57Kip2 expression in premalignant and malignant oral and laryngeal tissues. In particular, an
immunohistochemistry analysis of 79 cases of leukoplakia and 67 cases of squamous cell carcinoma (SCC) showed a p57<sub>Kip2</sub> decrease in leukoplakia and, more severely, in SCC (128). This study also showed a correlation between the worst 5-year survival rate and absence of p57<sub>Kip2</sub>. Subsequently, the same authors investigated 31 cases of leukoplakia and 109 laryngeal SCC, and confirmed their previous results (129). Very recently, miR-221 and miR-222 expression was found to be increased in oral carcinoma cells. Western blot analyses performed on samples of oral SCC indicated that p57<sub>Kip2</sub> (along with p27<sub>Kip1</sub>) is a target of miR action (130).

Lung cancers. Singhal and colleagues (131) used expression profiling to analyze the occurrence of cell cycle gene alterations in early-stage non–small cell lung carcinomas (NSCLC) and identified CDKN1C as a gene whose expression is selectively downregulated in lung cancer. In 2006, Pateras and colleagues (102) investigated p57<sub>Kip2</sub> content in 70 NSCLCs and identified a protein decrease (which was particularly evident in the nuclear compartment) in 89% of the cases. Functional studies showed that the reduction was due to methylation, allelic imprinting, and increased degradation (102).

Breast cancers. Hypermethylation of the CDKN1C gene was reported in 45% of breast cancer tumors (17 out of 38; ref. 132). In 2008, Larson and colleagues (133) analyzed 82 breast cancer samples for CDKN1C alterations. They showed that mRNA levels were reduced in 9 of 10 samples analyzed. p57<sub>Kip2</sub> protein staining was observed in 95% of normal epithelium, in 50% of carcinoma in situ, and in 28% of invasive carcinoma.

Hematological tumors. Several studies have been performed on CDKN1C expression in hematological cancers. In 1998, Iolascon and colleagues (134) reported an immunoblotting analysis of several cell cycle–related proteins showing that p57<sub>Kip2</sub> protein decreased in chronic myelogenous leukemia (CML). Thereafter, numerous investigations focused on the methylation status of CDKN1C gene. In 2002, Li and colleagues (135) analyzed lymphoid malignancies of B-cell phenotype. They observed an increase of CDKN1C promoter methylation. Thereafter, the status of the CDKN1C gene was investigated in 63 patients with newly diagnosed acute lymphoblastic leukemia (ALL) and 21 patients with relapse (136). The authors reported that CDKN1C hypermethylation was detectable in 50% of the first group and 52% of the second group. A subsequent investigation showed the importance of CDKN1C epigenetic changes as an independent marker of ALL (137). In the same year (2005), Gutiérrez and colleagues (138) reported a 7% CDKN1C hypermethylation in childhood ALL (74 samples analyzed). Of interest, they found that 53% of samples lacked the CDKN1C transcript, and the overall level was 8-fold lower than that observed in normal lymphocytes (P < 0.0001). Similarly, Canalli and colleagues (139) showed that aberrant DNA methylation of CDKN1C is a rare event in children with ALL (10% of 20 patients). Of interest, the protein level was not investigated in any of these studies. Subsequently, an evaluation of p57<sub>Kip2</sub> protein was performed in samples from 57 patients with ALL (140). The authors detected p57<sub>Kip2</sub> in 70% of the analyzed samples. We note, however, that of 15 patients with CDKN1C methylation, 10 expressed the protein and 5 did not. Thus, no direct correlation exists between the CDKN1C status and the protein level. Brakensiek and colleagues (141) reported the absence of CDKN1C methylation in myelodysplastic syndrome and acute myeloid leukemia (AML). A subsequent analysis of CDKN1C methylation in 50 AML and 25 ALL patients identified only 2 cases of AML and 1 ALL case with hypermethylated CDKN1C (142). Similarly, only 4 cases out of 56 newly diagnosed patients with chronic lymphocytic leukemias showed CDKN1C hypermethylation (143). The prognostic significance of CDKN1C methylation was recently studied in 44 patients with diffuse large B-cell lymphomas. The authors found CDKN1C methylation in 54.5% of the cases, but the epigenetic modification was not related to changes in chemoresponsiveness, disease-free survival, or progress of disease after chemotherapy (144). However, low-risk patients with CDKN1C methylation showed longer overall survival than patients with the same risk but no gene methylation. More recently, methylation of CDKN1C was detected in 53 out of 63 cases (84.1%) of diffuse large B-cell lymphoma, and the authors proposed the analysis of CDKN1C status for the detection of minimal residual disease (145).

Mechanisms of p57<sub>Kip2</sub> decrease in human tumors

The reduction of p57<sub>Kip2</sub> in cancers is generally due to a CDKN1C methylation and diminished expression, although posttranscriptional events have been described in some instances. Conversely, no CDKN1C mutations have been detected in cancer specimens. An increased methylation of the large CpG islands localized in the CDKN1C promoter represents the major mechanism of CDKN1C silencing. However, it should be noted that the gene methylation degree and the protein levels do not completely overlap. A second putative mechanism leading to p57<sub>Kip2</sub> downregulation is an increase in protein removal, mainly due to Skp2 overexpression in several cancers. However, the importance of Skp2-dependent degradation of p57<sub>Kip2</sub> is not completely clear, and how this event influences p57<sub>Kip2</sub> content is still a matter of debate. Several miRs (i.e., miR-25, miR-92b, miR-221, and miR-222) have been reported to control p57<sub>Kip2</sub> mRNA levels. In some instances, their upregulation has been associated with a decrease of p57<sub>Kip2</sub> in malignant cell lines and cancer specimens, including gastric, ovarian, colorectal, and hepatic carcinomas (23–25). However, miR-221 and miR-222 control a number of pivotal transcripts, including p27<sub>Kip1</sub>. Therefore, a superimposable decrease of p57<sub>Kip2</sub> and p27<sub>Kip1</sub> is expected in cancers where miR-221 and miR-222 are upregulated. Experimental data do not confirm this hypothesis, at least in ovarian cancers, where the upregulation of miR-221/222 affects only p57<sub>Kip2</sub> (25). Therefore, although circumstantial evidence suggests the great rele-
vance and general impact of miRs in cancers, further studies are necessary to substantiate their role in decreasing specific proteins.

Finally, level alterations of some transcription factors, such as p73 (activated during the DNA damage response) and LIT1, have been reported as the cause of CDKN1C-impaired activity. However, the available findings do not permit us to reach any conclusion about the relationship between the imprinting patterns of LIT1 and CDKN1C.

CDKN1C targeting and cancer treatment

The putative association between cancer phenotype and low content of p57kip2 suggests that the handling of p57kip2 levels might be advantageous for a target-oriented cancer therapy. A similar approach has been proposed for p27kip1 because the increase of this protein content might also be exploited for tumor treatment. However, there are vital differences in the likelihood of upregulating these 2 proteins. The levels and functions of p27kip1 are regulated mainly by removal of the protein and by its cellular localization. Both mechanisms appear difficult to handle, and thus the possibility of increasing p27kip1 levels or inducing its nuclear relocalization, albeit intriguing, seems hard to achieve pharmacologically. Conversely, as noted above, the level of p57kip2 is basically controlled by the degree of CDKN1C expression. In particular, the epigenetic status of the CDKN1C promoter (and more generally of the 11p15 region) and the level/activity of several transcription factors have been reported to strongly affect the expression of the gene. Thus, the increase of CDKN1C promoter activity appears to be an approachable target of therapy.

p57kip2 and demethylating agents. Growing evidence is implicating transcriptional silencing in the molecular pathogenesis of several human cancers. Epigenetic changes (defined as heritable alterations of gene expression without changes in the coding sequence) are the predominant mechanism of transcriptional silencing in tumors. The main epigenetic modification in humans is DNA methylation, and aberrant promoter methylation with associated gene silencing has been observed in many malignancies (146). CDKN1C silencing by promoter methylation has been reported to occur in several human cancer types. Treatment with demethylating agents, such as 5-azacytidine or 5-aza-2’-deoxycytidine, resulted in CDKN1C reexpression or overexpression in several experimental cancer models (105, 147–149). This finding suggests that compounds that reduce DNA methylation might be employed, possibly in association with other drugs, for the therapy of cancers with low p57kip2 content.

p57kip2 and HDAC inhibitors. It is now well established that HDACs are intriguing targets for cancer treatments. Histone acetylation is the main mechanism for modulating the nucleosome accessibility of DNA-dependent RNA polymerase, and histone hyperacetylation is a pivotal mechanism of gene silencing. Up to now, one HDAC inhibitor (HDACI), SAHA, has been approved for cutaneous T-cell lymphoma treatment (150, 151). Several other HDAC molecules (LBH589, SNDX275, PXD101, MGCD0103, oxamflatin, tubacin, romidepsin, and FK228) are in phase II–III clinical trials. Most of these HDACIs are being investigated in association with other anticancer drugs. The cancers that have been proposed as targets of novel HDACI treatments include both hematological malignancies (CML, AML, B-cell lymphoma, multiple myeloma, and Hodgkin and non-Hodgkin lymphoma) and solid tumors (breast, prostate, pancreatic, colorectal, thyroid, and non–small cell lung cancers).

HDACIs have multiple phenotypic effects due to changes in the acetylation pattern of both histone and nonhistone proteins. HDAC targets include proteins that are involved in the regulation of gene expression, the pathways of extrinsic and intrinsic apoptosis, cell cycle progression, redox pathways, mitotic division, DNA repair, cell migration, and angiogenesis.

Investigators have identified genes whose expression is modified by HDACI treatment by analyzing expression profiles before and after addition of the drug. Results obtained by the microarray approach have allowed the characterization of several HDACI effectors. One of these effectors, CDKN1C, has been identified as an early HDACI-modulated gene whose expression increases several-fold after HDACI treatment. This effect was observed in several cellular models (12, 115, 138). Some details of HDACI’s effect on the CDKN1C promoter were characterized recently. In particular, it was shown that histone hyperacetylation causes an increased binding of Sp1 to a sequence located in the interval from −87 to −113 bp of the CDKN1C promoter region (Fig. 1). The HDACI effect is particularly interesting in view of both the advanced status of approval for therapy with these molecules and the low levels of p57kip2 observed in several cancers.

Note that LBH589, an HDACI with a strong antimyeloma effect, is able to overcome resistance to dexamethasone, melphalan, and doxorubicin and to strongly upregulate CDKN1C expression (152). Moreover, it was shown that another HDACI molecule, romidepsin, specifically restored CDKN1C expression in rhabdoid tumor cells through promoter histone H3 and H4 acetylation, inducing cell cycle arrest (13). The proposed mechanism of romidepsin’s action was particularly complex. The authors suggested that the HDACI induces the expression of SMARC1, a transcriptional factor that is frequently lacking in rhabdoid cancers. SMARC1 then upregulates CDKN1C transcription, allowing the arrest in G0 of rhabdoid cell lines. By this series of events, romidepsin treatment recapitulates the effect of SMARC1 on CDKN1C expression.

p57kip2 and BCR-ABL inhibitors. CML is generally due to a balanced translocation between chromosome 9 and chromosome 21, with formation of the so-called Philadelphia chromosome. This novel chromosome carries the recombinant Bcr-Abl gene, which encodes for BCR-ABL. A large number of studies have shown that the recombinant chimeric kinase is responsible for the development of CML by constitutively activating several enzymes (e.g., Erk1/2,
AKT, Src, and Src-like kinases) and pathways that in turn stimulate proliferation and prevent apoptosis.

In the last few years, researchers have developed a highly specific inhibitor of the recombinant kinase, called imatinib mesylate. This molecule inhibits BCR-ABL, acting selectively as an ATP-competitive inhibitor, and it is able to shift an aggressive cancer into a stable chronic disease. In addition to imatinib, a second line of BCR-ABL inhibitors has been developed that includes dasatinib, nilotinib, and bosutinib. Although these drugs are more potent than imatinib, they are less specific for the BCR-ABL kinase. Some of these drugs (i.e., dasatinib and bosutinib) would be better considered as general tyrosine kinase inhibitors.

Imatinib and the other BCR-ABL inhibitors exert a significant antiproliferative effect on CML cells. A number of studies have shown that the mechanism of the imatinib antiproliferative action is, at least in part, associated with the upregulation of p27Kip1, due to both downregulation of Skp2 protein and p27Kip1 relocalization into the nucleus.

A recent study showed that all of the BCR-ABL inhibitors (i.e., imatinib, nilotinib, and dasatinib) are also able to induce upregulation of p57Kip2 by increasing the CDKN1C transcription (59). Of interest, the increase of p57Kip2 clearly preceded the accumulation of p27Kip1, at least in the CML cell lines investigated (59).

**Other drugs that increase p57Kip2 levels.** Although few other studies on drugs that increase p57Kip2 levels are available, 2 further examples must be described. It has been reported that a vitamin D3 analog, EB1089, inhibits the proliferation of human laryngeal squamous carcinoma cells by inducing the accumulation of p57Kip2. Of interest, the molecule is about 60-fold more potent than vitamin D3 and does not show significant hypercalcaemic effects (153). In addition, zoledronic acid, a bisphosphonate molecule endowed with interesting antiproliferative activity, has been reported to increase the level of p57Kip2 in leukemic cell lines (154).

**Future Directions**

The elevated mortality of p57Kip2 knockout mice highlights the relevance of this protein for correct embryogenesis. Similarly, substantial and timely changes of CDKN1C expression and p57Kip2 levels are required for an accurate differentiation of several tissues and the commitment of staminal cells toward specific phenotypes. Thus, the value of the Cip/Kip protein in eukaryotic physiology is indubitable.

Other evidence suggests that p57Kip2 participates in endoreduplication, apoptosis, and senescence. Moreover, the role of this protein in cytoskeletal organization highlights its significance in cell movement and substrate interaction. Stimulating findings suggest that p57Kip2 may regulate genome transcription by interacting with protein modulating gene expression. Finally, a significant number of studies underscore the importance of p57Kip2 downregulation in many human cancers.

On the other hand, the number of studies performed on p57Kip2 to date is fewer than 15% of those performed on p27Kip1, suggesting that our knowledge about this protein is still preliminary. For example, we have unsatisfactory data regarding p57Kip2’s metabolism, including the mechanism(s) of removal, cellular localization, posttranslational modifications, and interactors. It is important to emphasize that p57Kip2 protein has a more intricate domain organization than p21Cip1 and p27Kip1. The complexity of its sequence may be relevant for its function, particularly considering that p57Kip2 is intrinsically unstructured, a feature that suggests a remarkable flexibility and capability of interacting with several partners.

It is hoped that the elucidation of all these points will provide important avenues for investigating new aspects of carcinogenesis and developing further strategies for selective cancer treatments.

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No potential conflicts of interest were disclosed.

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