Subject Review

p57KIP2: “Kip”ing the Cell under Control

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Abstract

p57KIP2 is an imprinted gene located at the chromosomal locus 11p15.5. It is a cyclin-dependent kinase inhibitor belonging to the CIP/KIP family, which includes additionally p21CIP1/WAF1 and p27KIP1. It is the least studied CIP/KIP member and has a unique role in embryogenesis. p57KIP2 regulates the cell cycle, although novel functions have been attributed to this protein including cytoskeletal organization. Molecular analysis of animal models and patients with Beckwith-Wiedemann Syndrome have shown its nodal implication in the pathogenesis of this syndrome. p57KIP2 is frequently down-regulated in many common human malignancies through several mechanisms, denoting its anti-oncogenic function. This review is a thorough analysis of data available on p57KIP2, in relation to p21CIP1/WAF1 and p27KIP1, on gene and protein structure, its transcriptional and translational regulation, and its role in human physiology and pathology, focusing on cancer development. (Mol Cancer Res 2009;7(12):1902–19)

Introduction

Throughout the evolutionary process, cells have developed sophisticated molecular machinery in order to elicit, at any moment, the proper decision with respect to cell fate. For this reason, external and internal signals are continuously monitored, assimilated, processed, and/or integrated, via complex biochemical-signaling networks (1). At the heart of this complex, decision-making molecular machinery, lies a cell nucleus-located core that resembles, in its operation, a clock apparatus (1). The determination of the cell’s fate toward proliferation, arrest, differentiation, quiescence, or apoptosis relies upon this cell clock (1).

The decision for cell cycle progression to occur, which is synchronous with proliferation, requires timely activation, followed by subsequent ordered inactivation of cyclin-dependent kinases (CDK; ref. 1). CDKs are activated through the formation of heterodimers with cyclins, whose concentration alters periodically during the cell cycle. Sequentially coordinated stimulation of different CDK-cyclin complexes ensures proper cell cycle progression (1).

On the other hand, inhibitory effects over the activity of CDK-cyclin complexes may lead to cell cycle arrest (temporary or permanent), differentiation, senescence, quiescence, or apoptosis (1). Important negative regulators of CDKs are the CDK inhibitors (CKI).

There are two families of CKIs, the INK4 and the CIP/KIP family. The INK4 family includes p16INK4a (2), p15INK4b (3), p18INK4c (4), and p19INK4d (5, 6). The INK4 members are structurally related, having four ankyrin repeats. They cause G1 arrest through competition with cyclin D, thus preventing formation of active complexes of CDK4/6-cyclin D. The CIP/KIP family includes p21CIP1/WAF1/CDKN1A (7, 8), p27KIP2/CDKN1B (9, 10), and p57KIP2/CDKN1C (11, 12). The CIP/KIP family has a broader specificity for CDKs than the INK4 members. At low levels they bind to the CDK-cyclin heterodimer and promote their assembly, whereas at high levels they abrogate CDK activity. In this review we focus on the younger and least studied member of the CIP/KIP family, p57KIP2, presenting up-to-date evidence about the regulation of p57KIP2, its physiologic functions, and its impact in human pathology, focusing on carcinogenesis, in comparison with p21CIP1/WAF1 and p27KIP1.

Structure of the CDKN1C Gene and mRNA

The human CDKN1C gene resides in the telomeric end of chromosome 11, at the 11p15.5 locus (Fig. 1A; ref. 12). Mouse and rat p57KIP2 reside in the distal region of chromosomes 7 and 1, respectively (13, 14). The coding region of CDKN1C consists of four exons separated by three introns (Fig. 1A; ref. 15). p57KIP2 is rich in CpG islands both upstream and downstream of the putative transcriptional start site (16). Analysis of the CDKN1C promoter revealed the following consensus binding sites: (i) several Sp-1 (Stimulatory protein-1), (ii) ETS (erythroblastosis virus E26 oncogene), (iii) a TATA box [TBP (TATA box binding protein)], (iv) EGR1 (early growth response 1), (v) OCT1 (octamer-binding transcription factor 1), (vi) NFI1 (neurofibromin 1), (vii) two CAT elements, (viii) a GRE (glucocorticoid response element), (ix) a binding site for the p63 isoform ΔNp63α, and (x) binding sites for the Hes1 (hair and enhancer of split 1) and Herp2 (hes-related repressor protein 2) notch effectors (Fig. 1A; refs. 15, 17–21).

Alternative splicing of p57KIP2 intron 1 at different 3’ splice acceptor sites produces three transcripts (Fig. 1A). The major transcript corresponds to the one with the whole intron 1 spliced out (15). The splicing and expression pattern is conserved among humans and rodents (14).
Human and mouse p57KIP2 encode a 316- and a 335-amino acid protein, respectively, both migrating as 57 kD by SDS-PAGE electrophoresis (12). Mouse p57KIP2 protein consists of four distinct domains: (i) domain I (located in the amino-terminal region), a CDK inhibitory domain, sharing significant homology with the respective CDK inhibitory domain of p21CIP1/WAF1 and p27KIP1; (ii) domain II, a proline rich region; (iii) domain III, comprising an exceptional acidic repeat, in which every fourth amino acid is glutamic or aspartic acid; and (iv) domain IV (located in the carboxy-terminal region), a CDK inhibitory domain, sharing significant homology with the respective CDK inhibitory domain of p21CIP1/WAF1 and p27KIP1; (ii) domain II, a proline rich region; (iii) domain III, comprising an exceptional acidic repeat, in which every fourth amino acid is glutamic or aspartic acid; and (iv) domain IV (located in the carboxy-terminal region), a CDK inhibitory domain, sharing significant homology with the respective CDK inhibitory domain of p21CIP1/WAF1 and p27KIP1.
containing a conserved motif, named QT box (Fig. 1B). The QT box domain is significantly homologous with the carboxy-terminal domain of p27\textsuperscript{KIP1}. The human and mouse p57\textsuperscript{KIP2} amino- and carboxy-terminal domains are conserved, whereas the internal domains in the mouse p57\textsuperscript{KIP2} have been substituted in human p57\textsuperscript{KIP2} with a distinct domain rich in proline-alanine repeats, named the PAPA region (Fig. 1B; ref. 12). A putative nuclear localization signal (NLS) has been recognized in the QT box domain of the mouse p57\textsuperscript{KIP2}, similar to the NLS in the carboxy-terminal of mouse p21\textsuperscript{CIP1/WAF1} and mouse p27\textsuperscript{KIP1} (11).

Spectroscopic and hydrodynamic analysis indicate that human p57\textsuperscript{KIP2} lacks a stable helical and \(\beta\)-sheet structure, although the formation of a secondary structure cannot be precluded (22). p57\textsuperscript{KIP2} as well as p21\textsuperscript{CIP} and p27\textsuperscript{KIP1} are considered to be highly unfolded molecules (23, 24). Proteins that are fully or partially unfolded show a binding plasticity at the expense of thermodynamic stability, are degraded faster, and have an increased rate of evolution compared with folded proteins (25-27). CIP/KIP family members have a broad specificity for CDKs and are short-lived proteins that may be attributed to their internal regional structure.

The p57\textsuperscript{KIP2} Protein Interactions: Comparison with p21\textsuperscript{CIP1/WAF1} and p27\textsuperscript{KIP1}

p57\textsuperscript{KIP2} binds with CDKs in a cyclin-dependent manner (12). Association of p57\textsuperscript{KIP2}, with cyclins only, is very weak. p57\textsuperscript{KIP2} inhibits \textit{in vitro} the activity of the following CDK-cyclin complexes: CDK2-cyclin E, CDK2-cyclin A, CDK3-cyclin E, CDK4-cyclin D1, CDK4-cyclin D2, and to a lesser extent CDK1(CDC2)-cyclin B and CDK6-cyclin D2 (11, 12, 28). At low concentrations p57\textsuperscript{KIP2} can form active complexes with CDK2-cyclin A, enhancing their assembly, whereas at higher levels p57\textsuperscript{KIP2} inhibits the kinase activity of CDK2 (29). Similarly, CDK4-cyclin D1-heterodimer formation and activity is regulated in a p57\textsuperscript{KIP2} dose-dependent manner (28). This property is in accordance with the ability of both p21\textsuperscript{CIP1/WAF1} and p27\textsuperscript{KIP1}, at low concentrations, to promote

![Diagram of Chromosome 11 and Imprinting Control Regions](image_url)

**FIGURE 2.** Imprinting control regions of 11p15.5. The human chromosomal region 11p15.5 encompasses the \(H19\)-ICR (ICR-1) and the \(KCNQ1\)-KvDMR-ICR (ICR-2). \(H19\)-ICR is responsible for the imprinting status of several genes located in the telomeric end of 11p15.5 including \(H19\) and \(IGF2\). It is imprinted in the paternal allele (orange-dotted box), leading to the activation of the paternal \(IGF2\) and to the suppression of the maternal \(H19\). \(KCNQ1\)-KvDMR-ICR is located within intron 10 of the \(KCNQ1\) gene in the promoter region of a noncoding antisense transcript named \(KCNQ1\)-\(KvLQT1\). \(KCNQ1\)-KvDMR-ICR affects the imprinting status of the centromeric region of 11p15.5. The imprinting of the maternal ICR-2 (orange-dotted box) is associated with the suppression of the maternal \(LIT1\) and the expression of the maternal genes p57\textsuperscript{KIP2} and \(KCNQ1\). The arrows and the green boxes depict the active alleles, whereas the red boxes show the silent alleles.
the assembly of CDK4-cyclin D complex, whereas at higher levels to abrogate the kinase activity of the CDK-cyclin heterodimers (30-32).

The amino-terminal region of CIP/KIP members is necessary and sufficient for the inhibition of CDK-cyclin activity. It consists of three sites that are involved in the suppression of CDK-cyclin activity, a cyclin binding region, a CDK binding site, and a 310 helix (Fig. 1B). The 310 helix contains a phenylalanine-tyrosine pair of amino acids that is bound to the amino-terminal of CDK2, inside the catalytic cleft, mimicking the purine base of adenosine triphosphate (24). The 310 helix is indispensable for the *in vivo* inhibition of CDK2-cyclin A and CDK2-cyclin E by p57KIP2, but not for p21<sup>CIP1/WAF1</sup> and p27<sup>KIP1</sup> (29).

It has been proposed that the central region of p57KIP2 may serve as an important domain for protein interactions implicated in functions other than the CDK-inhibitory role (15). Indeed, the central region of mouse p57KIP2 interacts with the N-terminal of LIMK-1, a downstream effector of Rho (analyzed in the Cytoskeletal Organization section; ref. 33). The carboxy-terminus of p57KIP2 binds *in vitro* with proliferating cell nuclear antigen (PCNA) with a much lower affinity than p21<sup>CIP1/WAF1</sup> (34). In addition, p57KIP2 interacts with and inhibits the kinase activity of c-Jun NH<sub>2</sub>-terminal kinase/stress-activated protein kinase (JNK/SAPK) through its QT domain (35). Interestingly, p27<sup>KIP1</sup> does not inhibit JNK/SAPK activity, although it contains a QT domain (35), p21<sup>CIP1/WAF1</sup> also has the ability to bind and suppress the activity of JNK/SAPK via the amino-terminal domain (36). p57KIP2, but not p21<sup>CIP1/WAF1</sup> or p27<sup>KIP1</sup>, interacts both *in vivo* and *in vitro* through its amino-terminal domain with the transcription factor B-Myb (37). B-Myb competes with Cyclin A2 for the association with p57KIP2. Overexpression of B-Myb partially overwhelms the G1 cell cycle arrest induced by p57KIP2 in Saos-2 cells.

**Expression and Tissue Distribution Pattern of p57KIP2**

A highly cell-specific, temporal and spatial p57KIP2 expression profile has been found during the fetal, the postnatal, up to puberty, and the adult life cycle. Most of the data have been obtained from rodents (14, 38-44) and only sporadically in humans (11, 12, 44, 45). Specifically, the highest levels and most widespread tissue localization were observed during organogenesis. p57KIP2 is present in all major organs during embryonic development, and its levels of expression peaks at key stages of differentiation for each specific organ (14, 39, 41). Afterward, its expression declines to low or undetectable levels in several tissues (14, 39, 41), and only organs that mature after birth, such as testis (44), exhibit strong p57KIP2 staining in

![FIGURE 3. Methylation analysis of p57KIP2 promoter in normal and tumor human lung specimens. A. Methylation-specific PCR analysis shows promoter methylation in both normal (N) and tumor (T) lung tissue in a representative case. M+ and M- denote the methylation-specific PCR analysis with primers designed to bind specifically to methylated and nonmethylated CpG islands, correspondingly. B. Pyrosequencing analysis of the same representative case (depicted in A), showing decreased promoter methylation [assayed as methylation index (MtI)] in the tumor (T) versus the corresponding normal (N) tissue. Note the different scale in the y-axis of the pyrosequencing diagram.](image-url)
certain tissue compartments. Comparatively to prenatal tissues, fewer adult tissues continue to abundantly express p57KIP2 (11, 12). Nevertheless, an age-dependent p57KIP2 decline in some of these tissues has also been reported (42).

Human adult tissues expressing significant p57KIP2 mRNA levels are mainly skeletal muscle, brain, heart, lung, kidney, pancreas, testis, and placenta (11, 12); this is in contrast with the wider tissue distribution of higher levels of human p21cip1/waf1 and p27kip1 mRNA (11, 12). In rodents, p57KIP2 expression correlates, on average, with the human distribution pattern (12, 14, 39, 41), but a few species-specific exceptions do exist (14, 39, 41, 44).

Regulation of p57KIP2

Transcriptional Regulation of p57KIP2

Epigenetic control has emerged as an important mechanism for the transcriptional regulation of p57KIP2. The genetic locus of human p57KIP2, 11p15.5, harbors several imprinted genes (Fig. 2). Genomic imprinting refers to the differential epigenetic modulation of a gene leading to a parent-of-origin specific expression. This term means that the maternal and paternal allele of an imprinted gene display different properties, despite the fact that they have identical sequences. Epigenetic mechanisms include modulations of DNA methylation within CpG islands, chromatin remodeling (covalent modifications of histones), and silencing from various types of noncoding RNA transcripts (46). Methylation of DNA within CpG doublets, as well as methylation and deacetylation, in the amino-terminal tails of histones H3 and H4 are related with an inactive gene state, whereas the opposite is associated with gene activation. The cluster of imprinted genes in specific chromosomal regions, suggests that they share a common regulatory mechanism. The imprinting of such clusters is regulated by specific sequences acting in cis, known as imprinted control regions (ICR). ICRs encompass the following features: (i) a differentially methylated region (DMR) and chromatin conformation status between the maternal and the paternal genome and (ii) the establishment of the imprinting marks during gametogenesis, because this is the only developmental period in which the maternal and paternal genome are separated (47, 48).

The linkage of genes within the 11p15.5 region and the mouse homolog on distal chromosome 7 is conserved, showing the significance of the integrity of this region for the proper imprinting of the genes located therein (49-51). The regulation of imprinted genes in 11p15.5 is controlled by two ICRs, H19-ICR (ICR1) and KCNQ1/KsDMR-ICR (ICR2; Fig. 2). H19-ICR is located in the telomeric end of 11p15.5 and influences the imprinting of H19 and IGF2 by functioning as a chromatin insulator, restricting the access to shared enhancers (52). H19 is maternally expressed, whereas IGF2 is paternally transcribed. The KCNQ1-ICR coordinates the imprinting status of the more centromeric domain of 11p15.5 encompassing p57KIP2 and KCNQ1/KsLQT1 (53-56). p57KIP2 is maternally expressed, however there is a leaky expression from the paternal allele in most human tissues (57). The imprinted status of p57KIP2 is conserved between mice and human (13), p57KIP2 is hypomethylated in normal human kidneys (58), whereas we showed promoter methylation in normal human bronchial tissue (Fig. 3; ref. 59), suggesting the involvement of tissue-specific

FIGURE 4. Multiple roles of p57kip2 in cell biology. A. Positive p57kip2 immunostaining in mitosis (arrowhead) and in the nucleus (arrows) in the cancerous region of a representative case. B. Positive cytoplasmic p57kip2 immunostaining of a cancer cell (arrowhead) from a representative case. C. Putative role of p57kip2 in apoptosis and senescence.
mechanisms in the imprinting control of p57KIP2. Treatment with 5′-azacytidine, a demethylating agent, and/or trichostatin, a histone deacetylase inhibitor, activates p57KIP2 expression in mouse embryo fibroblasts, showing that both CpG methylation and histone deacetylase activities are involved in p57KIP2 imprinting (60). KCNQ1 is maternally expressed and harbors in intron 10 an antisense orientated nontranslated transcript named KCNQ1OT1/KvlQT1AS/LIT1 (long QT intronic transcript 1), which is paternally expressed (53-55). The KCNQ1-ICR coincides with the promoter of LIT1 in both human and mice (Fig. 2; refs. 53, 55, 61). The KCNQ1-ICR encompasses the typical characteristics of an ICR; it carries DNA methylation and acquires a repressive chromatin structure on the maternal allele, and the imprinting marking is established from the female germline (53-55, 62-64). The imprinting status of KCNQ1-ICR is associated with the expression pattern of the centromeric genes of 11p15.5, including p57KIP2. Specific regulatory elements within or in the flanking region of the KCNQ1 gene possibly act at a distance and regulate the expression of p57KIP2 according to the imprinting status of KCNQ1-ICR (65-70). The disruption of p57KIP2 imprinting in p57KIP2 transgenic lines supports the significance of cis-elements that are important for p57KIP2 expression lying at distance from the gene (71, 72). A functional role for LIT1 is also supported, participating in the establishment of the imprinting status of p57KIP2 (73-75), similar to the function of Xist on X chromosome inactivation (76) and of the noncoding Air on the IGF2R locus (77). In marsupials, LIT1 is expressed in the absence of p57KIP2 imprinting, suggesting a step-by-step evolutionary accumulation of these epigenetic mechanisms within the p57KIP2 region (78, 79).

Recently, a series of micro-RNAs (miR) have been reported to down-regulate the expression of p57KIP2, either specifically or along with other CIP/KIP members. Particularly, miR-221 and miR-222, which belong to the same cluster, are known from bioinformatic analyses to target both p57KIP2 and p27KIP2 (80-82). This effect has been validated in various human cell lines and in vivo in human hepatocellular and gastric carcinomas (80-83). In gastric cancer, p57KIP2 expression is also regulated by miR-25 (81), whereas in human embryonic stem cells it is specifically targeted by miR-92b (84). Functional analyses have shown that miR-dependent CIP/KIP silencing results in a shift in the number of cells from G1 to S-phase (80-82, 84). In murine embryonic stem cells and neural stem cells derived from embryonic stem cells, miR-106b and miR-17-92 suppress p57KIP2 and p27KIP2 and promote renewal of these stem cells (85).

Numerous other pathways control the expression of p57KIP2, possibly through activation of epigenetic mechanisms. In this context, the proteins SMARCB1 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1) and CTIP2 (chicken ovalbumin upstream promoter transcription factor interacting protein 2) up-regulate and correspondingly suppress p57KIP2 expression through chromatin remodeling (86, 87). Histone methyltransferase EZH2 can suppress p57KIP2 through histone H3 lysine 27 trimethylation (H3K27me3; ref. 88). Histone deacetylases inhibitors enhance the recruitment of Sp1 transcription factor in the promoter of p57KIP2, inducing its expression (89). The p53-related family member p73β induces the expression of p57KIP2 by activating the transcription of the nonsilent maternal allele (90). Interestingly, it was found that p73β isoform up-regulates the expression of both p57KIP2 and KCNQ1 without affecting the status of LIT1. p53 failed to induce p57KIP2, although it transcriptionally activates p21CIP1/WAF1 (12, 90, 91). The fact that p57KIP2−/− mice share a similar phenotype with p63−/− mice prompted Beretta and colleagues (17) to examine the possibility of a link between these two proteins. Indeed, the p63 isoform ΔNp63α binds to p57KIP2 promoter and induces its activation (17). The CDK inhibitor BMS-387032 up-regulates p57KIP2 through the transcriptional factor E2F1 in a direct manner (92). Induction of p57KIP2 after drug treatment, in turn, restrains E2F1 activity.
serving as a negative feedback loop. Transforming growth factor β1 (TGFβ1) induces \( p57^{kip2} \) in human hematopoietic cells, leading to cell cycle arrest (93). Silencing of \( p57^{kip2} \) abrogates the TGFβ1-induced decrease in proliferation of primary human limbal epithelial cells (94). TGFβ induces \( p27^{kip1} \) and \( p21^{cip1/waf1} \) as well (95, 96). Insulin-like growth factor 2 (IGF2) down-regulates \( p57^{kip2} \) in vivo in a dose-dependent manner (97). The Notch effectors Hes1 and Herp2 bind to and suppress the expression of \( p57^{kip2} \) (18, 19). Hes1 also directly suppresses the expression of \( p27^{kip1} \) (98), \( p27^{kip2} \), but not \( p21^{cip1/waf1} \), is induced by the transcription factor E47 that participates in human development, consistent with the unique role of this member of CIP/KIP family in embryogenesis (analyzed below; ref. 99). Interestingly, inhibitor of differentiation 2 (Id2), a natural inhibitor of E47, suppresses \( p57^{kip2} \). The chimeric protein PAX3-FOXO1, often present in rhabdomyosarcoma, reduces \( p57^{kip2} \) expression via the repression of the GC box-binding factor EGR1 (100). PLAG1/ZAC1 is an imprinted gene selectively expressed from the paternal allele from chromosomal region 6q24 (101). It has been shown that ZAC1 affects the expression of \( p57^{kip2} \) (101, 102). Human and mouse \( p57^{kip2} \) promoter contains a functional glucocorticoid response element (20). The induction of \( p57^{kip2} \) upon glucocorticoid treatment may be responsible for the antiproliferative effects of glucocorticoids (103). The antiproliferative effect of atrial natriuretic peptide may be partially mediated by the fact that it induces \( p57^{kip2} \) and \( p27^{kip1} \) as well (104). The vitamin D3 analog EB1089 inhibits the proliferation of the human laryngeal squamous cell carcinoma (Hep-2) through the induction of \( p57^{kip2} \) (105). Kido and colleagues (106) have shown that exon-2 of \( p57^{kip2} \) encodes a functional Bel1 response element, activated by the Bel1 trans-activator of the human foamy virus.

Post-translational Regulation of \( p57^{kip2} \)

Ubiquitilation and subsequent proteosomal degradation play a significant role in the regulation of \( p57^{kip2} \) protein levels. The SKP1-CUL1-F-box (SCF) complex is part of the ubiquitin-proteasome pathway and mediates ubiquitilation of target proteins from late G1 to early M phase. It consists of three constant subunits, named SKP1, CUL1, and RBX1, and a variable

FIGURE 6. Multiple epigenetic and genetic defects in the 11p15.5 chromosomal region are responsible for the phenotypic heterogeneity of Beckwith-Wiedemann Syndrome. Loss of imprinting of ICR-2 leading to the silencing of the \( p57^{kip2} \) gene accounts for the majority of cases with BWS. Uniparental disomy 11 (UPD11) represents a genetic abnormality because of abnormal chromosomal recombination leading to double dose of \( IGF2 \) and suppression of \( p57^{kip2} \). Loss of imprinting of ICR-1 causing double dose of \( IGF2 \) is a less frequent event in comparison with loss of imprinting of ICR-2 in BWS. Mutations in \( p57^{kip2} \), located mainly in the CDK-inhibitory or in the QT box/PCNA-binding domain, are implicated in the pathogenesis of BWS. In this figure we present the four most frequent mechanisms related to the pathogenesis of BWS. The sum of all above percentages is less than 100%, as other mechanisms (most of them are still unknown) are also responsible for the pathogenesis of BWS. The arrows and the green boxes denote active genes, whereas the red boxes show inactive genes.
component, an F-box protein that determines the target protein (107). The S-phase kinase-associated protein 2 (SKP2) belongs to F-box proteins. SKP2 interacts with p57KIP2 and promotes its degradation in a phosphorylation-dependent manner (108). SKP2 also mediates the degradation of p21CIP1/WAF1 and p27KIP1 (107). TGFβ1 stimulation induces p57KIP2 degradation via Smad-mediated transcription in serum-starved mouse osteoblasts (109, 110). The protein levels of p57KIP2 alter periodically during subsequent endoreduplications of trophoblast giant cells, diminishing before S-entry and reappearing after the completion of S phase (115). Introduction of a stable form of p57KIP2 into trophoblast giant cells blocks G1- to S-phase transition (115). A role of p57KIP2 in endoreduplication is reinforced by the fact that p57KIP2 triggers endoreduplication in mouse trophoblast stem cells by suppression of CDK1 activity (116). In the same study, p21CIP1/WAF1 and p27KIP1 could not compensate p57KIP2 for its role in endoreduplication of trophoblast giant cells (109).

### Functions of p57KIP2

#### Cell Cycle Control

The role of the CDK inhibitors p21CIP1/WAF1 and p27KIP1 in cell cycle arrest is well established (96, 113). In accordance with the other CIP/KIP family members, induction of p57KIP2 causes cell cycle arrest in G1 phase (11, 12). Transfection of SAOS-2 (sarcoma osteogenic, p53+/−, and Rb+/−) cells with the full-length p57KIP2 and a truncated p57KIP2*, in which the carboxy-terminal is omitted, led to G1 arrest (12). This finding is in agreement with the fact that the amino-terminal of p57KIP2 is indispensable for cell cycle arrest, through the inhibition of activity of the Cyclin-CDK complexes. In accordance with the above, induction of p57KIP2 in R-L/IB17 (subclone of the Mv1Lu mink lung epithelial cell line) caused cell cycle arrest in G1 phase (12). Endoreduplications are successive oscillations of G and S phases in the absence of mitosis and cytokinesis (114). The protein levels of p57KIP2 alter periodically during subsequent endoreduplications of trophoblast giant cells, diminishing before S-entry and reappearing after the completion of S phase (115). Introduction of a stable form of p57KIP2 into trophoblast giant cells blocks G1- to S-phase transition (115). A role of p57KIP2 in endoreduplication is reinforced by the fact that p57KIP2 triggers endoreduplication in mouse trophoblast stem cells by suppression of CDK1 activity (116). In the same study, p21CIP1/WAF1 and p27KIP1 could not compensate p57KIP2 for its role in endoreduplication in trophoblast stem cells. Moreover, p57KIP2 participates in the M-to-G1 transition through activation by p73 (100). Abrogation of p73 or its downstream effector p57KIP2 perturbs mitotic progression and transition to G1 phase (Fig. 4A; ref. 117).

### Table 1. Summary of the Results from the In vivo Studies of p57KIP2 Aberrations in Several Types of Cancer

<table>
<thead>
<tr>
<th>Type of Cancer</th>
<th>Alterations in p57KIP2</th>
<th>References</th>
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<tbody>
<tr>
<td>Wilms’ tumor</td>
<td>No mutations; frequent LOH in 11p15.5 (with a maternal bias) associated with decreased p57KIP2 mRNA; reactivation of the paternal p57KIP2 (LOI) in tumors with LOH; p57KIP2 expression varies</td>
<td>58, 212-217</td>
</tr>
<tr>
<td>Soft tissue sarcomas</td>
<td>No mutations; LOH with a maternal bias; increased mRNA expression of p57KIP2</td>
<td>213</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>ALL: Downregulation of p57KIP2 (increased methylation of p57KIP2 promoter is partially associated with its decreased expression); AML: increased methylation of p57KIP2 promoter; LMB: no mutations, increased methylation of p57KIP2 promoter</td>
<td>ALL: 219-222; AML: 16; LMB: 223</td>
</tr>
<tr>
<td>ALL, LMB</td>
<td>AC and GBM: Downregulation of p57KIP2 (increased methylation of p57KIP2 promoter)</td>
<td>219-222, 224, 225</td>
</tr>
<tr>
<td>Oral squamous cell carcinoma</td>
<td>No mutations; LOH with no parental bias; increased mRNA expression in cases with LOI; decreased positive immunostaining of p57KIP2 protein</td>
<td>126, 211</td>
</tr>
<tr>
<td>ALL</td>
<td>HC and EIC: no mutations; downregulation of p57KIP2 or alternatively reveal different regulatory mechanisms; limbal and trophoblast stem cells. Moreover, p57KIP2 participates in the M-to-G1 transition through activation by p73 (100). Abrogation of p73 or its downstream effector p57KIP2 perturbs mitotic progression and transition to G1 phase (Fig. 4A; ref. 117).</td>
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<tr>
<td>Lung carcinomas (non-small cell lung carcinomas)</td>
<td>No mutations; frequent LOH with a maternal allele bias; increased methylation of p57KIP2 promoter (and in a few cases upregulation of LIT1) related with decreased expression of p57KIP2; faint p57KIP2 positive immunostaining in tumor versus normal tissues; diminished p57KIP2 protein status associated with increased SKP2 expression</td>
<td>59, 227, 228</td>
</tr>
<tr>
<td>Esophageal squamous carcinoma</td>
<td>p57KIP2-positive immunostaining at relatively high frequency in tumors</td>
<td>126</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>No mutations; increased levels of miR-25, miR-221, and miR-222 (target p57KIP2)</td>
<td>219-222; 224, 225</td>
</tr>
<tr>
<td>PC and IPMN</td>
<td>PC: p57KIP2-positive immunostaining; PC: no mutations; LOH, loss of heterozygosity; LOH, loss of imprinting; IPMN: Decreased transcriptional and translational levels of p57KIP2 associated with promoter hypermethylation</td>
<td>PC: 231, 230; IPMN: 247</td>
</tr>
<tr>
<td>HC and EIC</td>
<td>HC: no mutations; downregulation of p57KIP2 related to loss of LIT1-ACR methylation; reduced positive immunostaining of p57KIP2 in cases with high biological aggressiveness; downregulation of p57KIP2 protein associated with upregulation of miR-221; EIC: Decreased expression of p57KIP2 in cases with biological aggressive phenotype</td>
<td>HC: 15, 80, 232-236; EIC: 237</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>Decreased positive immunostaining of p57KIP2 in colorectal carcinomas</td>
<td>15, 238, 239, 249</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>No mutations; decreased expression of p57KIP2 mRNA correlated in some cases with LOH; decreased expression of p57KIP2 protein</td>
<td>15, 88, 227, 240</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>No mutations; Increased methylation of p57KIP2 promoter; decreased transcriptional levels and diminished positive immunostaining of p57KIP2 protein</td>
<td>15, 88, 227, 240</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>Intense positive immunostaining of p57KIP2; decreased positive immunostaining in cases with high biological aggressive phenotype</td>
<td>241, 242</td>
</tr>
<tr>
<td>Malignant adenocortical tumors</td>
<td>Reduced mRNA expression of p57KIP2</td>
<td>243, 244</td>
</tr>
<tr>
<td>Thyroid carcinomas</td>
<td>Decreased p57KIP2 positive immunostaining in cases with biological aggressive phenotype</td>
<td>245</td>
</tr>
</tbody>
</table>

Abbreviations: AC, astrocytoma; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; EIC, extrahepatic bile duct carcinoma and intrahepatic cholangiocarcinoma; GBM, glioblastoma; HC, hepatocellular carcinoma; IPMN, intraductal papillary mucinous neoplasm; LMB, lymphoid malignancies of B-cell origin; LOH, loss of heterozygosity; LOI, loss of imprinting; PC, pancreatic carcinoma.
Cytoskeletal Organization

p57KIP2 has a nuclear distribution, consistent with its anti-proliferative capacity (11). However, it has been shown that p57KIP2 may accumulate in the cytoplasm participating in the regulation of the cytoskeletal dynamics (33). Mouse p57KIP2 binds, through its central domain, to LIM-kinase-1 and translocates it from the cytoplasm to the nucleus, without abrogating its kinase activity. LIM-kinase-1 is a serine-threonine kinase that phosphorylates and inactivates an actin depolymerization factor cofillin, leading to the stabilization of actin stress fibers (118). Our laboratory provided evidence for the specificity of human cytoplasmic p57KIP2 localization in normal and cancerous material from lung (Fig. 4B; ref. 59). p21CIP1/WAF1 and p27KIP1 also have a distinct function in the cytoplasm, different from the cyclin-CDK inhibitory role. They have the ability to regulate the Rho signaling pathway, therefore interfering with cytoskeletal organization (119). Altogether it seems that the CIP/KIP members participate in the interconnection between cell cycle control and cytoskeletal organization.

Putative Role of p57KIP2 in Apoptosis and Senescence

p57KIP2 enhances staurosporine-induced apoptosis in human cervical adenocarcinoma cell line (HeLa) cells in a CDK-inhibitory independent manner (120). p57KIP2 translocates to mitochondria and activates the intrinsic apoptotic pathway (121). Targeted silencing of p57KIP2 suppresses the p73 mediated apoptosis in the lung cancer cell line H1299 and in the colorectal carcinoma cell line HCT116 caused by cisplatin treatment (122). Upregulation of p73 in postmitotic hNT neurons induces p57KIP2 and BAX leading to apoptosis (123). In contrast, p57KIP2 suppresses JNK/SAPK mediated apoptosis in the human embryonic kidney cell line HEK293 after exposure to UV light, demonstrating an anti-apoptotic role of p57KIP2 (35). Similarly, induction of p57KIP2 by BMS387032 has a strong anti-apoptotic effect on the MDA-MB-231 cell line (92). A dual role in apoptosis has been documented for p27KIP1 and p21CIP1/WAF1, although most reports confer, especially for p21CIP1/WAF1, an anti-apoptotic role (95, 119, 124). We have not found an association of p57KIP2 or p27KIP1 with apoptosis neither in vivo in non-small cell lung cancer specimens nor in vitro in the A549 lung cancer cell line (59, 125). The role of CIP/KIP members in apoptosis may be tissue specific, dependent on the intracellular localization and the pathway they target (Fig. 4C). CIP/KIP members also play a role in cellular senescence. The induction of p57KIP2 in human astrocytoma cell lines restrains their proliferation and induces a senescent phenotype (126). Similarly, upregulation of p57KIP2 in the PPC-1 prostate cancer cell line induces a senescence-like phenotype (127). However, more studies are required to clarify the relation of p57KIP2 with cellular senescence (Fig. 4C), in contrast to the well-established role of p21CIP1/WAF1 (96).

Lessons from Mice

Mice lacking p57KIP2 (p57KIP2−/−) display increased postnatal mortality accompanied by severe developmental abnormalities (Fig. 5; refs. 128-130). Mice with targeted disruption of p57KIP2 exhibit limb shortening owing to abnormal endochondral ossification, cleft palate, omphalocoele, gastrointestinal tract defects, adrenal cortex enlargement, renal medullary dysplasia, abnormalities in lens fiber cells, and increased body weight (128-131). Surviving mice exhibited severe growth retardation, immaturity of testes and uterus, and vaginal atresia (130). Mice with targeted deletion of the maternal p57KIP2 allele showed similar phenotypic abnormalities with p57KIP2−/− mice (128, 129). The observed developmental defects occur possibly because of delayed differentiation and increased apoptosis. Excess p57KIP2 in mice leads to increased embryonic lethality and to decreased body size, suggesting that embryonic growth is sensitive to p57KIP2 dosage (131). Endochondral bone formation is compromised in null mice for either parathyroid hormone-related peptide (PTHrP) or p57KIP2, in an opposite manner (128, 129, 132, 133). In PTHrP/p57KIP2−/− mice, the absence of p57KIP2 partially normalizes the PTHrP-null phenotype (133). Mutant mice lacking either both p57KIP2 alleles or only the maternal one, with the paternal allele intact, display severe placental abnormalities including trophoblastic dysplasia (134). Diminution of p57KIP2 expression in mouse placenta did not alter the activity of both CDK2 and CDK4, providing evidence for a biological role of p57KIP2 in placenta, different from the inhibition of the CDK activity, p21CIP1/WAF1 knockout mice (135, 136) and p27KIP2 null mice (137-139) do not exhibit gross developmental abnormalities, revealing the importance of p57KIP2 in embryonic development in relation with the other CIP/KIP members (Fig. 5). Interestingly, mice lacking both p21CIP1/WAF1 and p57KIP2 exhibit all the phenotypic alterations caused by p57KIP2 null mice alone, but also express novel abnormalities in lung, skeleton, and skeletal muscles, demonstrating the cooperation between these proteins in mouse development (38). The frequency of embryonic lethality increases in p57KIP2−/− and p27KIP1−/− mice compared with p57KIP2−/−/− and p27KIP1−/−/− mice (140). Moreover, p27KIP1−/− and p57KIP2−/− mice exhibit more severe developmental defects in placenta and lenses than p27KIP1−/− mice alone, showing the significance of p57KIP2 in development. Immunohistochemical analysis of p57KIP2 and p27KIP1 in certain tissues from mice revealed a mutual pattern of expression, suggesting the importance of both KIPs for proper development (39). Susaki and colleagues (141) created a knock-in mouse model in which the p57KIP2 gene was replaced with p27KIP1 gene. In this model the mice are viable and phenotypically healthy, revealing the partial functional redundancy between the KIP members.

The Role of p57KIP2 in Cooperation with p21CIP1/WAF1 and p27KIP1 in Development

The fact that p57KIP2 is expressed during mouse embryogenesis in several anatomical structures underlies its role in embryonic development (128, 129). Specifically, p57KIP2 is found in derivatives of all three germ layers, the ectoderm, mesoderm, and endoderm, and in all major organs of the developing fetus (14, 39, 41). The earliest expression in rodents is between E9.0 and E10.5 in the heart (14, 39, 41). As mentioned above in the Expression and Tissue Distribution Pattern of p57KIP2 section, the highest p57KIP2 expression coincides with the stage of differentiation for each specific organ (14, 39, 41). Importantly, the strongest p57KIP2 expression appears at the interface of developing tissues with different embryonic origin, as in the direct contact between epithelial and mesenchymal...
tissues (41). Comparative analyses of all three CIP/KIP family members during rodent prenatal development have revealed a complex spatial and temporal pattern of expression (38-40, 43, 140). Of note, cyclin D3, a p57KIP2 inhibition target, also exhibits a specific temporal and spatial expression pattern in differentiating mammalian tissues, as well as in relation to the other cyclin D members (142). Yet, with a few exceptions, no systematic correlation has been done with the corresponding p57KIP2 temporal and spatial pattern of expression.

p57KIP2 cooperates with negative (p27KIP1) and positive (CDK2, CDK4, cyclin D, and cyclin E) cell cycle regulators to promote lens fiber differentiation (140, 143, 144). Particularly, it has an opposite effect on the proliferating function of cyclin D3 (144). p57KIP2 and p27KIP1 participate in mouse retina development through the induction of cell cycle exit in the retinal progenitor cells, but in mutually different subpopulations (145). Downregulation of p57KIP2 and p27KIP1, through Notch signaling, inhibits mouse lens epithelia differentiation (19, 146). p57KIP2 has two distinct roles in mouse retina, it controls proliferation of progenitor cells and promotes the development of amacrine interneurons (147). In addition, p57KIP2 promotes cell cycle exit of precursor cells in zebrafish retina through its activation by the Sonic hedgehog (Shh; ref. 148).

p57KIP2 participates in the development of the nervous system (149). It collaborates with p27KIP1 on the regulation of neuronal migration of mouse cortex (150) and rat neurogenesis (151); it promotes oligodendrocyte differentiation (152, 153); and is required for the maturation of dopamine neurons (154). Downregulation of p57KIP2 mediates in vitro myelination by Schwann cells (155).

p57KIP2 participates in myogenesis (38). It stabilizes MyoD, a muscle-specific transcription factor involved in myogenic differentiation, through the inhibition of the phosphorylation-dependent degradation by cyclin E-CDK2 complex and by direct association of the amino-terminal domain of p57KIP2 with the basic helix-loop-helix domain of MyoD (156-158). In turn, MyoD up-regulates p57KIP2 in a p73-dependent manner (159). MyoD induces p21CIP1/WAF1 in a direct manner (158). Notably, cyclin D3 also participates in striated muscle cell differentiation (142), but no link has been established with p57KIP2.

Interestingly, as podocytes progress to terminal differentiation they are characterized by an increase in both p57KIP2 and cyclin D3, along with a decrease in the levels of cyclin D1 (160).

Differential immunohistochemical staining of p57KIP2 in human placenta cells suggests a role of p57KIP2 in human placenta development (161). The coordinated expression of the CIP/KIP members promotes keratinocyte differentiation (162, 163). During pancreatic development p57KIP2 controls both self-renewal and cell cycle exit of pancreatic progenitors (18). p57KIP2 and p27KIP1 cooperate in a temporal manner for pituitary development; p57KIP2 promotes cell cycle exit of progenitors, whereas p27KIP1 prevents cell cycle reentry (164). p57KIP2 also implicated in hepatocyte maturation (40), T-lymphocyte cell development (165), spermatogenesis and Leydig cell development (45), hematopoiesis and hematopoietic stem cell cycle arrest (93, 166), chondrocyte differentiation (133, 167), and adrenal cortex development (168).

Overall, CIP/KIP members possibly share partial overlapping roles in embryogenesis, although p57KIP2 seems to have the upper hand over p21CIP1/WAF1 and p27KIP1 in development. The strong impact of p57KIP2 on embryo survival and development, along with its more complex internal structure relative to its counterparts, which seems to keep differentiated organs under control postnatally and in adulthood, implies that p57KIP2 may have preceded the appearance of the other CIP/KIP members evolutionarily.

p57KIP2 in Pathology

We analyze here the impact of p57KIP2 on human pathology, apart from its role in carcinogenesis, which is examined separately below in the Role of p57KIP2 in Carcinogenesis section.

Several studies have shown the role of p57KIP2 in the pathogenesis of Beckwith-Wiedemann Syndrome (BWS; ref. 169). BWS is a clinically heterogeneous disease, with most patients exhibiting macrosomia, macroglossia, omphalocele, and a predisposition to pediatric tumors, including Wilms’ tumor, hepatoblastoma, adrenocortical carcinoma, and rhabdomyosarcoma (170). The phenotypic heterogeneity of BWS reflects the different epigenetic and genetic defects affecting the 11p15.5 domain (Fig. 6; refs. 171-174). The most frequent alteration is loss of imprinting of the paternal LIT1 locus (1C2)-2, leading to biallelic expression of LIT1, which is associated with reduced levels of p57KIP2 (53-55, 62, 173, 175, 176). Epigenetic alterations in H19-ICR, related with upregulation of IGF2, is a less frequent event (177). Patients with BWS exhibit mutations of p57KIP2, more frequently seen in familial BWS (30-50%), than in sporadic BWS (5-10%; ref. 177). p57KIP2 mutations are mainly detected in the CDK-inhibitory domain and in the QT box domain (172, 173, 178-182). Mutations in the CDK-inhibitory domain abrogate the CDK2-inhibitory activity, whereas mutations in the QT box domain retain the capability of CDK2 suppression, but exclude the protein from the nucleus, therefore in both cases the cell cycle inhibitory activity is compromised (181, 182). Mosaicism of paternal isodisomy of 11p15.5 [paternal uniparental disomy 11 (pUPD11)], accounts for almost 20% of BWS cases (177). Uniparental disomy results from chromosomal rearrangements owing to abnormal recombination occurring in meiosis, mitosis, or successive meiosis and mitosis (183). Tissues employing pUPD11, have two paternal but no maternal copies of 11p15.5, exhibiting double dose of the growth-promoting paternally expressed IGF2 and diminished expression of the growth-inhibiting maternally expressed gene p57KIP2 (46, 184). The existence of a predominant pUPD11 distribution in the abdominal organs of two fetuses with BWS exhibiting omphalocele (185) is reminiscent of the p57KIP2-M phenotype in mice (129). Double mutant mice with loss of function of p57KIP2 and with gain of function of IGF2, mimicking pUPD11, display several similarities with BWS phenotype (186).

Mutant mice, defective only for the maternal p57KIP2 allele (p57KIP2−/−), display the whole spectrum of the clinical manifestations of pre-eclampsia including proteinuria and elevated blood pressure (134, 187). The observed trophoblastic hyperplasia may be the leading cause (188). Further studies, failed to recapitulate pre-eclampsia manifestations in the particular...
mouse model, although they invariably exhibited placenta abnormalities, suggesting the significance of environmental factors in the development of this disease (188, 189). Recently, Romanelli and colleagues (190) reported the existence of three women with haemolysis, elevated liver enzymes, and low platelets (HELLP)-preeclampsia who gave birth to children with BWS possessing mutations in p57KIP2. Therefore, a possible role of the imprinted gene p57KIP2 in the pathogenesis of preeclampsia is suspected, although further studies are needed to clarify this issue. p57KIP2 is absent in androgenic complete hydatidiform moles, an abnormality of the trophoblast proliferation, in which the total genome of the fetus is paternally derived (161). Therefore, p57KIP2 has been shown to be a highly sensitive marker for the differential diagnosis of complete from partial hydatidiform mole (191-197).

Evidence supports a role for p57KIP2 in cardiovascular pathology. The expression of p57KIP2 is increased, whereas p21CIP1/WAF1 and p27KIP1 decline in both acute and end-stage heart failure, reversing to the fetal pattern of expression (198). A microsatellite polymorphism, GTn repeat, positioned in the promoter region of p57KIP2, is related with atherosclerosis and myocardial infarction (199). The presence of the lower repeats was significantly more frequent among healthy individuals. Longer GTn repeats may lead to decreased transcription of p57KIP2, conferring increased susceptibility to atherosclerosis. Forced expression of p57KIP2 in cardiomyocytes from transgenic mice exerts a protective role from ischemia-reperfusion injury, possibly via the suppression of the JNK/SAPK pathway (200). p57KIP2 and p27KIP1 are decreased in damaged podocytes from patients with focal segmental glomerulosclerosis in comparison with controls (201). This finding is in agreement with previous studies in experimental models in mice and rat showing that p57KIP2 is suppressed in glomerular diseases (160). Of note, p57KIP2 downregulation is accompanied by a similar decrease in cyclin D3 and increase of cyclin D1 (160, 202). p57KIP2 seems to be involved in the pathogenesis of autosomal dominant polycystic kidney disease (203). Focal hyperinsulinism is caused by the absence of the maternal 11p chromosome (204). The fact that p57KIP2 is lost, whereas IGFR2 is up-regulated in tissue specimens from patients with focal hyperinsulinism compared with controls, may explain the increased β-cell proliferation in focal hyperinsulinism (205).

Role of p57KIP2 in Carcinogenesis

Studies in Mice and Cell Lines

Takahashi and colleagues (130) did not detect the development of spontaneous tumors during a 5-month observation period of p57KIP2 null mice. However, in p57KIP2−/− mice, 6 months postgrafting, the prostate grafted under the kidney capsule of athymic mice developed prostatic intraepithelial neoplasia, a precursor to prostatic cancer (206). Similarly, p27KIP1/WAF1−/− mice did not develop tumors until the 7th month of age, whereas after 16 months they were malignant prostate (207). p27KIP1−/− mice exhibit an increased risk of pituitary tumorigenesis (137-139). p57KIP2 is down-regulated during immortalization of cultured human mammary epithelial cells (HMEC; ref. 208). The HMECs undergoing the immortal conversion initially lost the p57KIP2 maternal allele, associated with the upregulation of the previously silent allele; however, after adoption of the fully immortal phenotype, the remaining allele was also downregulated, but not lost. Ectopic expression of p57KIP2 in Ewing cells induces G1 arrest (21). p57KIP2−/− P-mouse embryo fibroblasts (P signifies parthenogenetic, exclusively comprising the maternal genome) exhibit increased proliferation in comparison with P-mouse embryo fibroblasts, where p57KIP2 is intact (209). We showed that restoration of p57KIP2 levels in A549 restrains cell cycle proliferation, without any significant effect on apoptosis (59). Similarly, exogenous expression of p57KIP2 in leukemia cells suppresses cell growth (210). Moreover, p57KIP2 induces apoptosis only in leukemia cell lines with methylated p57KIP2. p57KIP2−/− induced expression leads to the adoption of the senescence phenotype in astrocytoma cell lines and PPC-1 as mentioned earlier (126, 127). Overexpression of p57KIP2 reduces the invasiveness of U373 human malignant glioma cells independent of the extracellular matrix (211).

p57KIP2 is frequently down-regulated in human cancers through several mechanisms

p57KIP2 is frequently down-regulated both transcriptionally and translationally in many human cancers, and its decreased expression is correlated with aggressiveness in several malignancies (Table 1; refs. 15, 16, 58, 59, 80, 81, 88, 126, 212-249). Two studies examining the p57KIP2 status in Wilms’ tumor, show that p57KIP2 loss of heterozygosity was associated with reactivation of the silent allele (loss of imprinting), possibly reflecting the activation of a homeostatic feedback mechanism to prevent cell growth, similar with the p57KIP2 expression pattern in the immortalization of HMECs (208, 215, 216). Similarly, the increased immunostaining of p57KIP2 in esophageal squamous cell carcinoma and its positive association with cyclin E and Ki-67 may reflect the activation of a feedback mechanism to limit the increased proliferation (246). In the same manner, in colorectal carcinogenesis p57KIP2 levels increase significantly from normal mucosa to adenomas and ultimately decrease in primary carcinoma (248). Downregulation of p57KIP2 is inversely correlated with proliferation in non-small cell lung carcinoma (59), in pancreatic adenocarcinoma (230), in hepatocellular carcinoma (234), in extrahepatic bile duct carcinoma, and intrahepatic cholangiocellular carcinoma (237). Diminished expression of p57KIP2 predicts poor prognosis in oral squamous cell carcinoma (225), in laryngeal squamous cell carcinoma (226), in hepatocellular carcinoma (234, 235), and in malignant ovarian tumors (242). Recently, Yang and colleagues (88) provided evidence that decreased p57KIP2 expression is related with poor outcome in breast cancer. The fact that we have not observed any association between p57KIP2 or p27KIP1 immunostaining status, respectively, and patient stage in non-small cell lung carcinoma suggests a relatively early inactivation of both KIP members in the development of this type of cancer (59).

The above data, showing that p57KIP2 increases in the initial stages of cancer followed by a gradual decrease as malignancy progresses, imply that a selective pressure leading to its loss takes place (59, 208, 246, 248). As we have previously shown, the DNA damage response (DDR) pathway, which is also activated from the earliest stages of cancer (250, 251),
seems to follow the same fate (252). Having in mind that p57KIP2 is a target of p73β (90) and that DDR regulates p73β, as we have recently shown (253), it is tempting to speculate that p57KIP2 could represent an additional downstream DDR effector. Such a scenario should be further validated.

No mutations of p57KIP2 have been found in all cancer specimens analyzed so far (Table 1). In accordance with p57KIP2, p21CIP1/WAF1 and p27KIP1 are rarely mutated in human cancers (96, 113). Therefore, other mechanisms are implicated in p57KIP2 downregulation in human cancers (Table 1). Our analysis on 70 matched normal tumor specimens from patients with non-small cell lung carcinoma revealed that multiple mechanisms lead to p57KIP2 downregulation in lung cancer (59). The majority of cases had decreased expression of p57KIP2, which was strongly associated with increased SKP2 levels and reduced transcriptional levels of p57KIP2. Increased methylation of p57KIP2 promoter and loss of heterozygosity were correlated with p57KIP2 mRNA downregulation. Ablant methylation and histone modifications in the p57KIP2 locus seem to be a crucial mechanism for the inactivation of p57KIP2 in common malignancies (16). The reactivation of the p57KIP2 gene after treatment with demethylating agents and histone deacetylase inhibitor in many cancer cell lines denotes the significance of the epigenetic mechanisms in p57KIP2 downregulation in several human cancers (16, 59, 86, 89, 210, 223, 241, 249, 254-256).

We showed that treatment of the A549 cell line with 5′-aza-cytidine, a demethylating agent, up-regulates p57KIP2 mRNA levels significantly, whereas the protein levels do not increase in a significant manner (59). Only demethylation along with SKP2 siRNA-silencing restored p57KIP2 protein levels in A549 cells. Interestingly, in our study, a subset of cases with increased p57KIP2 mRNA exhibited decreased p57KIP2 protein levels possibly because of the increased SKP2 levels. Therefore, it is essential to examine the p57KIP2 mRNA levels along with its protein status in order to clarify cumulatively the end effect from the demethylating agents on gene transcription and from p57KIP2 protein degradation in human cancer. It is possible that increased p57KIP2 mRNA expression, observed in some malignancies, may be counterbalanced by elevated p57KIP2 protein degradation (Table 1). In view of emerging molecular therapeutic approaches, aiming to restore the CIP/KIP activity in various malignancies, such collective information is crucial for their success (257).

According to the imprinting pattern of LIT1 and p57KIP2, the expression status of these genes is inversely correlated. However, we did not observe a strong inverse relation between LIT1 and p57KIP2 expression, which is in accordance with the findings of Hoffmann and colleagues (249) in bladder carcinoma, Sato and colleagues (247) in pancreatic ductal neoplasms, and Nakano and colleagues (258) in several colorectal cancer cell lines. It is possible that LIT1 cooperates with several elements lying in the 11p15.5 region, in order to promote the proper imprinting of p57KIP2. The absence of a significant relationship between p57KIP2 and LIT1 expression status may be due to the disruption of the integrity of the 11p15.5 region due to loss of heterozygosity, which is a frequent event in this chromosomal region in many cancers (259). Alternatively, this mechanism may be tissue specific. Soejima and colleagues (260) found that diminution of p57KIP2 in several human esophageal cancer cell lines was associated with the epigenetic status of LIT1 locus and not of its own promoter.

A functional link between miRNA-25, miRNA-221 and miRNA-222, and p57KIP2 mRNA levels, mediating suppression of the latter, has been recently shown in HeLa; in human breast cancer cell line; in the human gastric cancer cell lines SNU-638, AGS, and MKN-28 (81); in K562 (human leukemia cell line); and in the T98G (human glioblastoma) cell line (82), as well as in vivo in human gastric and hepatocellular carcinomas (80, 81).

It seems that p57KIP2 has a more clear-cut oncosuppressor activity in comparison with the other members of CIP/KIP family so far. p57KIP2 is frequently targeted in most cancers supporting its tumor-suppressor activity, however deductions about p57KIP2 function can be made only in the tissue context examined. Indeed, p21CIP1/WAF1 has both an oncogenic and a tumor-suppressor activity dependent on the tissue studied (96). Moreover, p27KIP1 harbors an oncogenic function when it is localized in the cytoplasm of many common tumors (119). The cytoplasmic localization of p57KIP2 in some cancers may introduce an oncogene-like behavior. However, the fact that cytoplasmic mouse p57KIP2 translocates LIMK1 from the cytoplasm to the nucleus, and that the elevated cytoplasmic LIMK1 promotes cancer invasion (261), supports a tumor-suppressor activity for cytoplasmic p57KIP2, although more studies are needed to clarify this issue.

The examination of the unique central domain of p57KIP2, PAPA region, by Tokino and colleagues (15) resulted in an association of specific PAPA polymorphisms with breast cancer risk. However, in a subsequent analysis in a larger cohort by Li and colleagues (262), this correlation was not confirmed. Therefore, this issue remains unresolved and requires further investigation.

**Conclusion**

p57KIP2 has a distinct role in embryogenesis among CIP/KIP members. The increased mortality of p57KIP2 knockout mice denotes its impact on tissue development. The absence of mutations of p57KIP2 in all cancers studied so far implies the importance of the integrity of this gene for cell viability. The participation of p57KIP2 in cell cycle arrest and the identification of putative novel functions in endoreduplication, mitotic exit, apoptosis, senescence, and cytoskeletal organization highlight its significance in the context of its frequent downregulation in many human cancers. The effort to unravel the biology of p57KIP2 has already provided a useful diagnostic marker in the differential diagnosis of complete from partial hydatidiform moles. In the future, the putative antitumor functions of p57KIP2 may be exploited to design novel therapeutic agents for BWS and several common malignancies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Notes Added in Proof**

The following publications have come to our attention since the review entered the proof preparation.

1. Vlachos and Joseph reported that human p57KIP2 interacts in the cytoplasm with LIMK-1 enhancing its kinase activity,
resulting in the inactivation of cofilin. They showed that the cytoplasmic localization of p57KIP2 negatively affects cell mobility, reinforcing the oncosuppressor activity of p57KIP2 in the cytoplasm (263).

2. Mascarenhas and colleagues depicted the involvement of p57KIP2 in hematopoietic development (264).

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Molecular Cancer Research

p57KIP2: "Kip"ing the Cell under Control

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