Transcriptional regulation of $p57^{kip2}$ expression during development, differentiation and disease

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1. ABSTRACT

$p57^{kip2}$ is the most complex member of the CIP/KIP family of cyclin-dependent kinase inhibitors and plays a fundamental role in regulating cell cycle and differentiation during mammalian development. Consistently with a key role for $p57^{kip2}$ in the spatial and temporal control of cell proliferation, its expression is fine-tuned by multiple regulatory mechanisms, resulting in a tissue-, developmental phase- and cell type-specific pattern. Moreover, $p57^{kip2}$ is an imprinted gene, further supporting the importance of its proper expression dosage. Importantly, misregulation of $p57^{kip2}$ expression has been associated, more frequently than mutations in its coding region, to human growth disorders, such as Beckwith-Wiedemann and Silver-Russell syndromes, as well as to the onset of several types of cancers. This review will summarize the molecular mechanisms regulating $p57^{kip2}$ transcription during differentiation and development, their relationship with the imprinting control and their alterations in growth-related diseases and cancer. Particular attention will be given to the role of epigenetic mechanisms, involving DNA methylation, histone modifications, long-range chromatin interactions and non-coding RNAs in modulating and integrating the functions of cis-regulatory elements and trans-acting factors.

2. INTRODUCTION

$p57^{kip2}$ (hereafter referred to as p57), also called Cdkn1c, is a member of the Cip/Kip family of cyclin-dependent-kinase (CDK) inhibitors (CKIs),...
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which also includes the related members p21<sup>cip1</sup> (p21) and p27<sup>kip1</sup> (p27) (1, 2). The N-terminal region includes a cdk-inhibitory domain sharing sequence homology with p21 and p27. The C-terminal region includes a QT domain, involved in protein-protein interactions and containing several phosphorylation sites. This domain shows similarity with the C-terminal region of p27 whereas it is absent in p21. The intervening region is unique to p57 and, unlike the N- and C-terminal domains, is poorly conserved between mouse and human (3, 4).

p57 plays a key role in regulating cell proliferation and differentiation during mammalian development. Its best established function is to block cell cycle progression through the inhibition of G1/S CDKs, a function required not only for limiting the cell numbers, but also for promoting cell decisions towards differentiation (5). However, increasing evidence indicates that p57, like other CKIs, influences cellular and developmental processes also by additional and multiple biochemical mechanisms independent of CDK inhibition. For example, p57 can directly regulate differentiation by interacting with some lineage-specific transcription factors, such as MyoD in skeletal muscle cells (6), Nurr1 in dopaminergic neurons (7), Mash1, NeuroD and Nex/Math 2 in neural stem cells (8). Moreover, a fraction of p57 is localized in the cytoplasm and interacts with the actin cytoskeleton-modifying enzyme, LIM-kinase 1 (9). The p57 effects on cytoskeletal dynamics have been involved in the regulation of cancer cell migration (10), neuronal motility (11) and mitochondria-mediated apoptosis (12). Recently, p57 has also been involved in the response to intracellular or extracellular stresses by eliciting apoptosis and senescence (13).

The different phenotypes displayed by knock-out mice indicated that p57, among Cip/Kip family members plays the most critical role in mouse development. In fact, while mice knock-out for p21 or p27 are viable, despite the presence of several phenotypic defects and increased tumor incidence (14, 15), the ablation of p57 results in neonatal mortality (16–18). This outcome is likely the consequence of the multiple developmental abnormalities affecting kidney, adrenal gland, lens, palate, skeleton, intestine, abdominal muscle and placenta. Interestingly, the replacement of p57 with p27, in a knock-in mouse model, resulted in the recovery of most of the developmental defects present in p57 knock-out mice, except in kidney, placenta, and abdominal wall (19). Thus, while the permanence of a few phenotypic alterations implies that some specific functions of p57 cannot be performed by p27, the correction of most of the defects, observed when p27 is expressed following the same spatial and temporal pattern as p57, highlights the importance of the peculiar p57 regulation during particular developmental phases and in specific tissues. Although p57 is also expressed in some tissues and organs in adult animals, little is known about its functions after development, due to the early lethality caused by its knock-out. Until now, the conditional ablation of p57 has been realized only in the brain and in the hemopoietic system (20, 21). Both studies suggested a role for p57 in the function of progenitor and stem cell populations.

Considering that p57 is a key regulator of cell cycle, apoptosis and cell motility, it is not surprising that p57 loss has been found in many human malignancies, which is why it is commonly recognized as an oncosuppressor (22–24).

As described below, p57 shows a tissue-restricted and cell type-specific expression pattern, involving complex regulatory mechanisms that act at multiple levels, from chromatin to post-translational modifications. This complexity probably reflects the need for the organisms to maintain the proper levels of this CKI in the right place and at the right time during development. In this context, an abnormal p57 function has been found to account for several important diseases affecting cell growth and development. Remarkably, most of the p57-associated disorders have been ascribed to unbalanced gene expression more frequently than to gene mutations.

As expected for a developmentally regulated gene, the expression of p57 is subject to epigenetic mechanisms of control, involving DNA methylation and several types of chromatin modifications, so referred because of their ability to initiate and maintain active or repressive states of gene expression through cell divisions.

Importantly, p57 is subject to genomic imprinting, a process whereby a subset of genes, most of which regulate embryonic growth and development, are monoallelically expressed in a parent-of-origin-specific manner (25). The evolutionary significance of the parental specificity of silencing is still a matter of debate. However, the occurrence of a tight and sophisticated regulation of this process suggests that the expression dosage of imprinted genes is of critical importance. p57 is paternally imprinted, that is it is expressed almost exclusively from the paternal allele, in both human and mouse (26, 27). In this regard, a considerable amount of information has been accumulated on the epigenetic mechanisms that lead to the paternal-specific silencing of p57 and on the detrimental effects of the altered dosage of its levels after loss of imprinting. More recently, a great effort is being spent in the study of the multi-layered regulation of p57 expression during development and differentiation.

In light of the peculiar genomic context in which the p57 gene is located, this review will be focused on the molecular mechanisms regulating p57
transcription during differentiation, their relationship with the imprinting control and their alterations in growth diseases and cancer. For a description of the mechanisms of post-transcriptional regulation and for further details on p57 structure and functions see the review by Pateras and coworkers (28) and the cited references.

3. EXPRESSION PATTERN OF p57

CKIs display differential expression patterns, both in vivo and in in vitro cell culture systems, indicating that they are regulated by distinct embryonic mechanisms, presumably in order to exert specific functions in different cell contexts.

A general picture, based on the in vivo distribution of the mRNA and/or protein products, is that p21 is mainly expressed in the differentiated cells of adult tissues (29), while p27 and p57, the two structurally closest members of the Cip/Kip family, are widely expressed in most developing tissues (30, 31). However, despite their co-expression in several embryonic regions, p27 and p57 also show mutually exclusive patterns among organs, tissues and cell populations. In fact, while p57 expression prevails in intestine, cartilage, skeletal muscle, palate and pancreas, p27 expression prevails in thymus, spleen, retina, testis and ovary (30). Moreover, the two CKIs are differentially expressed in distinct populations of neural progenitors as observed in retina, spinal chord and cerebral cortex (11, 32, 33). Another important distinctive feature of the two CKIs is that, while the expression of p27 persists at high levels throughout the adult life, the expression of p57 declines significantly around the birth age, becoming restricted to subsets of organs, tissues and cell types. In adult mice and humans, p57 expression is detectable in heart, brain, lung, kidney, pancreas, skeletal muscle, testis and placenta (3, 4, 17, 18, 30).

p57 not only accumulates in terminally-differentiated, post-mitotic cells but also in some types of undifferentiated, quiescent stem cells. In particular, p57 mRNA and protein levels are high in adult quiescent hematopoietic stem cells (HSCs) and decline during their differentiation into progenitors (21). Similarly, in the adult mouse hippocampus, p57 protein is expressed in quiescent neural stem cells (NSCs), but not in the proliferating progenitors (20). All these findings denote that p57 plays a dual role in the delicate balance between proliferation and growth arrest, one concerning the coupling between cell cycle exit and differentiation, and the other one the self-renewal activity of adult stem cells.

The expression pattern of p57 in in vitro cell systems reflects at least in part the pattern observed in vivo. p57 transcription is upregulated during differentiation of skeletal myoblasts (34–37), podocytes (38), oligodendrocytes (39) epidermal keratinocytes (40, 41) and cerebral cortical precursors (42).

As described below, studies based on the manipulation of signaling pathways, transcription factors and epigenetic modifiers shed some light on the regulatory networks and molecular mechanisms converging on the transcriptional control of p57.

4. SIGNALING PATHWAYS

On the basis of studies in cell culture, while p21 is generally recognized as the target of checkpoint pathways and p27 as the mediator of contact inhibition of cell proliferation, p57 appears to come into play downstream to development/differentiation signals.

Various extracellular stimuli and intracellular pathways have been reported to up- or downregulate p57 transcription, depending both on the specific pathway and on the cell-type context. Although the picture emerging is very complex due to the occurrence of indirect effects resulting from the cross-talk between the different pathways, some direct functional links between external cues and p57 regulation have been clearly established for Notch and TGF-β signaling.

The Notch/Hes pathway is highly conserved across species and plays key roles in coordinating cell proliferation and development in a variety of tissues (43). Notch-dependent repression of p57 has been reported for several cell types, such as pancreatic progenitors (44), lens fiber cells (45), intestinal crypt cells (46, 47), hepatocellular carcinoma cells (48), liver progenitors (49) and skeletal muscle cells (50). The picture emerging from these studies is that Notch activity represses p57 in order to maintain undifferentiated progenitor cells in a cycling status until the occurrence of an overcoming differentiating signal that promotes p57 expression, growth arrest and differentiation.

Similarly, Wnt/β-catenin signaling, another well-known pathway promoting cell proliferation during development (51), also inhibits p57 expression. This effect was noticed in several cell types, such as neural precursors (52, 53), hematopoietic stem cells (54), corneal epithelial stem cells (55) and adrenocortical cancer cells (56) . However, the functional role of p57 repression downstream of the Wnt/β-catenin pathway has not been investigated yet.

In contrast to Notch/Hes and Wnt/β-catenin, TGF-β/Smad signaling, a widespread and multifunctional pathway (57), upregulates p57 expression in cell contexts in which it exerts a cytostatic effect. In this regard it has been demonstrated that p57-dependent cell cycle arrest mediates the ability of TGF-β to induce and/or maintain the quiescence of hematopoietic stem and progenitor cells (58–61). Moreover, the
focused on p57. In differentiating muscle cells, p57 transcription is induced by the myogenic transcription factor MyoD, a muscle-specific bHLH protein (34, 35, 37, 70, 71), while in undifferentiated muscle progenitors it is inhibited by Hes1 and Hey1, two related bHLH Notch-effectors (50). Hes1 represses p57 also in intestinal cells, participating in the maintenance of the proliferative crypt compartment of the small intestine (46, 47), in pituitary cells, preventing their inappropriate cell cycle exit during pituitary development (72), and in pancreatic β-cells, contributing to their in vitro de-differentiation (72, 73). bHLH-dependent regulation of p57 has also been noticed in different cell types of the nervous system, in particular: in Schwann cells, in which p57 is a specific target of the tissue-restricted bHLH protein Mash-2 (74), in differentiating neuronal progenitors, in which the upregulation of p57 requires the ubiquitous bHLH protein TCF4/E2–2 (75), and in neuroectodermal cell lines, in which p57 expression is induced by the ubiquitous bHLH protein E47 and suppressed by the HLH protein Id2, a natural inhibitor lacking the basic domain (76). The observation that the hypoxia inducible factors of the HIF family, required for the low oxygen-dependent upregulation of p57, are also bHLH proteins, further supports a widespread role for bHLH factors in mediating p57 regulation in response to different types of signals (Table 1).

As reported below, most of the mentioned bHLH proteins have been found to directly interact with E-box or E-box-like sequences within different types of p57 regulatory elements.

### Table 1. bHLH proteins involved in p57 transcriptional regulation

<table>
<thead>
<tr>
<th>bHLH</th>
<th>Effect on p57</th>
<th>Cell system</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hes1</td>
<td>repression</td>
<td>Muscle cells</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intestinal cells</td>
<td>(46, 47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic progenitors</td>
<td>(44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic β-cells</td>
<td>(73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatocellular carcinoma cells</td>
<td>(48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pituitary progenitors</td>
<td>(72)</td>
</tr>
<tr>
<td>Herp2</td>
<td>repression</td>
<td>Lens fiber cells</td>
<td>(45)</td>
</tr>
<tr>
<td>MyoD</td>
<td>induction</td>
<td>Muscle cells</td>
<td>(34, 35, 37, 50, 70, 71, 100–102)</td>
</tr>
<tr>
<td>Mash2</td>
<td>induction</td>
<td>Schwann cells</td>
<td>(74)</td>
</tr>
<tr>
<td>TCF4/E2–2</td>
<td>induction</td>
<td>Neuronal progenitors</td>
<td>(75)</td>
</tr>
<tr>
<td>Id2 (HLH)</td>
<td>repression</td>
<td>Neuroectodermal cells</td>
<td>(76)</td>
</tr>
<tr>
<td>E47</td>
<td>induction</td>
<td>Neuroectodermal cells</td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic β-cells</td>
<td>(83)</td>
</tr>
<tr>
<td>HIF1α/HIF2α</td>
<td>induction</td>
<td>Hematopoietic stem cells</td>
<td>(63–65, 131)</td>
</tr>
<tr>
<td>HIF1α</td>
<td>induction</td>
<td>Growth plate chondrocytes</td>
<td>(66)</td>
</tr>
</tbody>
</table>

upregulation of p57 by other members of the TGF-β superfamily has been linked to the induction of differentiation, as reported for Bone Morphogenetic Proteins BMP2 and BMP6 in epidermal keratinocytes (40) and for BMP11/GDF11 in developing spinal cord (62).

Although p57 function has been primarily linked to development/differentiation signals, this CKI has also been proposed as an effector of other cellular pathways. p57 is induced downstream of the Hypoxia/HIF-1 pathway and its upregulation correlates with cell cycle arrest in hematopoietic stem cells growing under hypoxic conditions (63–65) and in hypoxic chondrocytes during bone development (66). Moreover, a recent research showed that p57, like p21, is induced by genotoxic stress, and activates the G1/S checkpoint while promotes cell survival (67). This finding expands our understanding of the intracellular pathways and mechanisms underlying the tumor suppressor function of p57.

### 5. TRANS-ACTING FACTORS AND CIS-REGULATORY ELEMENTS

Important insight on how extracellular signals and intracellular pathways impact on p57 transcription has been provided from studies focused on basic Helix Loop-Helix (bHLH) transcription factors. bHLH proteins are a large superfamily of transcriptional regulators, recognizing target sites termed E-box sequences and exerting activating or repressing functions. bHLH proteins play critical roles in regulating gene expression downstream of cell signaling events and in coordinating differentiation with growth arrest (68). Originally highlighted for p21 (69), the ability of bHLH factors to regulate CKI expression was subsequently focused on p57. In differentiating muscle cells, p57 transcription is induced by the myogenic transcription factor MyoD, a muscle-specific bHLH protein (34, 35, 37, 70, 71), while in undifferentiated muscle progenitors it is inhibited by Hes1 and Hey1, two related bHLH Notch-effectors (50). Hes1 represses p57 also in intestinal cells, participating in the maintenance of the proliferative crypt compartment of the small intestine (46, 47), in pituitary cells, preventing their inappropriate cell cycle exit during pituitary development (72), and in pancreatic β-cells, contributing to their in vitro de-differentiation (72, 73). bHLH-dependent regulation of p57 has also been noticed in different cell types of the nervous system, in particular: in Schwann cells, in which p57 is a specific target of the tissue-restricted bHLH protein Mash-2 (74), in differentiating neuronal progenitors, in which the upregulation of p57 requires the ubiquitous bHLH protein TCF4/E2–2 (75), and in neuroectodermal cell lines, in which p57 expression is induced by the ubiquitous bHLH protein E47 and suppressed by the HLH protein Id2, a natural inhibitor lacking the basic domain (76). The observation that the hypoxia inducible factors of the HIF family, required for the low oxygen-dependent upregulation of p57, are also bHLH proteins, further supports a widespread role for bHLH-dependent upregulation of p57, are also bHLH proteins, further supports a widespread role for bHLH factors in mediating p57 regulation in response to different types of signals (Table 1).
Additional trans-acting factors, have been characterized in different experimental settings (Figure 1).

### 5.1. Promoter elements

A proximal promoter element of mouse \( p57 \), highly conserved in humans and extending from \(-165 \) to \(+15 \) bp from the transcriptional start site, is the target of different regulatory factors. In reporter gene assays, this region is responsive to TGF-β (60), MyoD and p73 (37). However, the absence of E-box elements and of binding sites for the TGFβ- effectors Smad, as well as for p73, is consistent with the occurrence of indirect transactivation mechanisms. In fact, it has been recently shown that the TGF-β-dependent induction of \( p57 \) is mediated by the transcription factor GATA2 (58). Moreover, the \( p57 \) proximal promoter contains multiple binding sites for the ubiquitous factors Sp1 and Egr1, which we demonstrated to mediate its MyoD-dependent transactivation in muscle cells (34). This pathway involves the up-regulation of Egr1 and the formation of a complex of Sp1 with MyoD-induced p73, which results in the recruitment of both Sp1 and Egr1 to the promoter. The observation that \( p57 \) is a target of Sp1 (77) and Egr1 (78–80) also in different types of human cells, further reinforces the importance of these factors in the promoter function. The \( p57 \) proximal promoter also contains several recognition sites for CTIP2/Bcl11b, a transcriptional repressor involved in several developmental processes and cancer (81). Accordingly, CTIP2/Bcl11b has been reported to repress \( p57 \) expression in human tumor cells by directly interacting with its binding sites and recruiting chromatin modifying enzymes on the promoter (77, 82). Importantly, the \( p57 \) proximal promoter, as the entire locus, is rich in potentially methylable CG dinucleotides (Figure 2A). This feature underlies a complex interplay between DNA methylation and transcription factor binding in the modulation of \( p57 \)-promoter activity (see below).

Unlike the proximal promoter, distal promoter sequences contain several E-box and E-box-like motifs which have been shown to be directly involved in \( p57 \) regulation by some bHLH proteins. For example, TCF4/E2–2 upregulates \( p57 \) in neuronal cells by interacting with a group of E-boxes located between \(-1300 \) and \(-2300 \) bb from the transcriptional start site (75). Similarly, in pancreatic β-cells, the induction of \( p57 \) involves the binding of E47 to a group of E-boxes located between \(-4000 \) and \(-3000 \) bp (83). Moreover the Notch effector Hes1 represses \( p57 \) by binding to a site located at \(-3300 \) in intestinal cript progenitor cells (46) and the Hes-related repressor protein Herp2 through a site located at \(-4200 \) bp in proliferating lens epithelial cells (45).
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Other types of regulatory elements and transcription factors play a part in the activation of $p57$ promoter upon various stimuli. One of them is the glucocorticoid receptor that binds to a glucocorticoid response element, located at about $-5000$ bp and conserved in mouse and human, and mediates the antiproliferative effect of dexamethasone in human tumor cells (84). Another example is provided by the Smad1/Atf2 complex that stimulates $p57$ expression in mouse fibroblasts by interacting, upon DNA damage, with a promoter region extending from $-2000$ to $-700$ bp (67).

5.2. Enhancer elements

In contrast to proximal and distal promoter elements, much less is known about more distant regions, potentially acting as enhancers and responsible for the maintenance of high $p57$ levels in specific tissues. Previous studies based on $p57$ expression from bacterial artificial chromosome (BAC) transgenes, bearing large regions of the mouse locus, suggested that tissue-specific enhancers are located far downstream of the gene (85). In particular, sequences required for full $p57$ expression in skeletal and cardiac muscle, cartilage, cranial ganglia, intestine and palate were inferred to lie between the positions $+25$ kb and $+260$ kb downstream of the gene. More recent studies, based on the integration of data relative to the sequence conservation between human, mouse and rat, to the genomic distribution of chromatin marks typical of enhancers and to the phenotypic effects of chromosomal rearrangements, identified three candidate enhancer sequences for human $p57$ expression (86, 87). These putative enhancers are located at about positions $+55$ kb, $+97$ kb and $+350$ kb, respectively. The most distal of the three elements has been shown to be bound by the CCCTC-binding factor (CTCF) and to be in contact with $p57$ promoter through a constitutive chromatin loop in human placenta (87). However, the exact role of the observed long-range interaction has not been addressed yet. Interestingly, a muscle-specific enhancer, placed in a position similar to the most proximal of the three proposed enhancer elements, has been shown to regulate $p57$ expression in mouse skeletal muscle cells. This enhancer is activated by the myogenic factor MyoD in differentiating myoblasts, while it is repressed by the Notch effector Hes1/Hey1 in muscle progenitors, through direct binding of either factor to a group of E-box sequences (50).

5.3. Imprinting control region

A distant regulatory region controls $p57$ imprinting, through complex cis-acting mechanisms leading to the epigenetic silencing of the paternal allele. The $p57$ gene is located within a conserved cluster of imprinted genes on human chromosome 11p15.5., a region conserved and syntenic with the distal part of mouse chromosome 7 (88, 89). This region contains two imprinting domains associated with the Beckwith-Wiedemann syndrome (BWS), a human overgrowth disorder characterized by multiple
developmental defects and predisposition to cancer (88, 90–92) (see also below). The first one, the Igf2/H19 domain, includes Igf2, coding for a member of the insulin family of polypeptide growth factors and H19, which is transcribed in a long non-coding RNA (lncRNA). The second one, generally referred to as the Kcnq1/Kcnq1ot1 domain, includes, in addition to p57 and other six imprinted genes, also Kcnq1 and Kcnq1ot1 (Figure 1). Kcnq1 encodes a subunit of a voltage-dependent potassium channel while Kcnq1ot1 is transcribed in a macro lncRNA. In both human and mouse, Kcnq1 and p57 are paternally imprinted, that is they are preferentially expressed from the respective maternally-derived alleles, while Kcnq1ot1 is maternally imprinted. Genetic studies in humans and mice led to the identification of an imprinting control region, called ICR2 or KvDMR1, whose deletion caused loss of imprinting of the genes of the kcnq1 domain (93, 94). KvDMR1 was defined as a 2.6 kb region in human and a 2.8 kb region in mouse, mapping about 150 kb downstream of p57, and bearing differential epigenetic marks on the two parental chromosomes (see also below). The cis-acting repressive effect of KvDMR1 on the imprinting domain involves both the enhancer-blocking activity of this region, mediated by the insulator factor CTCF, and the chromatin silencing by the macro lncRNA Kcnq1ot1, whose promoter is comprised within KvDMR1 (95). A number of excellent reviews address in more detail the molecular mechanisms underlying the imprinting control of the Kcnq1/Kcnq1ot1 domain (see for example (96–99)).

While the best established role of KvDMR1 is the allele-specific silencing of the domain, our recent work in skeletal muscle cells demonstrated that this region can also control the expression of the maternal, non-imprinted p57 allele (100–102). The observation that, during differentiation, p57 was co-induced with the co-imprinted gene Kcnq1 hinted at the possible involvement of KvDMR1, a regulatory region shared by the two genes, in their coordinate upregulation. Most importantly, we found that, prior to differentiation, KvDMR1 participates in a repressive long-range chromatin interaction with p57 promoter, mediated by CTCF. Upon differentiation, the myogenic factor MyoD binds to E-box-like sequences located near CTCF binding sites within a specific KvDMR1 sub-region, causing the release of the chromatin loop and the induction of maternal p57 expression. The use of multiple strategies by a single transcription factor, such as MyoD, for regulating p57 expression (induction of trans-acting factors targeting the promoter, activation of an enhancer and modification of the chromatin architecture at KvDMR1) probably reflects the necessity of counteracting the multiple blocks that assure a tight control of p57 transcription.

The findings obtained in muscle cells highlighted the composite nature of KVDMR1 and revealed a novel role of this element in regulating p57 expression, distinct from the imprinting control. The involvement of KvDMR1 in the differentiation-dependent regulation of p57 has been demonstrated only for muscle cells at this moment. However, the observation that p57 is co-induced with Kcnq1 not only by MyoD in muscle cells (102) but also by p73 (103) and by E47 (76) in other cell types, is consistent with a general involvement of KvDMR1 in the regulation of the domain also independently of imprinting.

It is important to mention that p57 belongs to so-called “Imprinted Gene Network”, a regulatory system involving the frequently coordinate expression of a number of both maternally and paternally imprinted genes, which promote or restrict cell proliferation (104, 105). This co-regulation seems to implicate trans-regulatory interactions between imprinted genes. In particular, two members of the network have been involved in fine-tuning in trans the expression of at least a subset of imprintined genes, by affecting their normally expressed alleles. Zac1, a zinc-finger transcription factor encoded by the maternally imprinted gene Zac1/Plag1, directly regulates the activity of an enhancer of the imprinted genes Igf2 and H19 and of the promoter of Kcnq1ot1 (106, 107). H19, a long non-coding RNA encoded by the paternally imprinted gene H19, recruits chromatin modifying complexes and promotes histone modifications on the differentially methylated regions of Igf2, Slc38a4 and Peg1. The molecular mechanisms of the transcriptional control of p57 in this context are still unclear. This issue, extremely fascinating in the field of the developmental role and regulation of imprinted genes, undoubtedly deserves further investigation.

6. CHROMATIN MODIFICATIONS

Numerous studies highlighted the existence of a complex interplay between trans-acting factors and chromatin modifications at the promoter, the gene body and the imprinting control region of p57. Particular attention has been paid to the roles of DNA methylation and post-translational modifications of core histones. More recently, an increasing number of studies are being focused on the dynamics of the spatial organization of the p57 locus and on the functional interaction of the lncRNA Kcnq1ot1 with chromatin.

6.1. DNA Methylation

The p57 gene is almost entirely included in a CpG island that starts about 600 bp upstream of the transcriptional start site and extends into the gene body. In mouse, but not in human, the p57 CpG island is differentially methylated between parental chromosomes, with the paternal allele hypermethylated and the maternal allele hypomethylated (108–110). It has been suggested that this differential methylation functions as a secondary imprinting mark, acquired
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post-implantation during development and involved in maintaining and/or reinforcing the paternal p57 repression imposed by the imprinting control region (108).

Complete methylation of p57 promoter sequences has been observed also in human cancers and tumor cell lines, in which p57 is silenced (see below). Interestingly, in undifferentiated skeletal myoblasts, in which p57 has not yet been induced, the promoter seems biallelically hypermethylated and inaccessible to the MyoD-regulated factors Sp1 and Egr1, which recognize preferentially unmethylated CG-rich sequences (34). During MyoD-driven differentiation, p57 promoter undergoes a DNA demethylation process, consequent to the MyoD-dependent release of the chromatin loop with KvDMR1, which allows the binding of the above factors and the upregulation of the gene (34, 102). The finding that p57 induction only occurs from the maternal allele indicated that the observed demethylation concerns the normally active allele and is not related to the imprinting regulation. It would be interesting to verify the occurrence of a similar process in other differentiating cell types.

The regulatory factors and the molecular mechanisms modulating the DNA methylation status of the genome, although they are the object of extensive investigation (111, 112), are still poorly understood. Even less is known about the dynamics of DNA methylation at p57 promoter. Concerning the imprinted p57 allele, it was reported that Lsh, a chromatin remodelling protein regulating DNA methylation, directly binds to p57 promoter and is required for the maintenance of hypermethylation of the paternal allele and for its silencing (113). Interestingly, this mechanism appeared to be specific for p57 promoter and not for other imprinted loci. It would be appealing to explore the possible role of Lsh in the hypermethylation of the maternal allele in undifferentiated or in cancer cells.

While numerous data indicate that promoter DNA methylation plays a significant part in p57 silencing, the role of this modification in the control of p57 transcription is much more complex, since DNA methylation also affects the function of the KvDMR1 CpG island. In fact, the DNA methylation mark on the imprinting control region exerts an opposite effect on p57 expression with respect to the DNA methylation mark on the promoter (Figure 2B). KvDMR1 hypermethylation, which is normally present on the maternal allele, correlates with p57 expression, while its hypomethylation, present on the paternal allele, correlates with p57 silencing. Two regulatory strategies, not necessary mutually exclusive, have been proposed to explain the methylation-dependent effect of KvDMR1 on p57 transcription. The first one would involve the enhancer blocking function of a KvDMR1 sub-region, which requires the binding of the insulator factor CTCF to two unmethylated recognition sites (114). The other one would involve the cis-repressive action of the macro lncRNA Kcnq1ot1 (see below), whose transcription starts just within KvDMR1 and is promoted by its hypomethylation as well.

The complex relationship between DNA methylation and p57 expression is also supported by the contrasting effects of the genetic ablation of individual members of the DNA methyltransferase (DNMT) family, the enzymes catalyzing the transfer of methyl groups to cytosines. In particular, the lack of DNMT3a or DNMT3L, required for de novo methylation of most imprinting control regions in the germline, results in the biallelic silencing of p57 (115–117). This effect can be ascribed to the failed methylation of Kcnq1ot1 promoter and to the consequent biallelic expression of the macro IncRNA. In contrast, the lack of DNMT1, the enzyme involved in maintaining the methylation pattern of CpGs during DNA replication, results in the biallelic expression of p57 (108, 109, 118). This result can be explained by the failure to keep hypermethylated the paternal p57 promoter. Interestingly, the conditional depletion of DNMT3a in muscle precursors has been recently reported to cause loss of promoter methylation and p57 upregulation (119). Consistently with this finding, not only DNMT1 (120), but also DNMT3a (119) has been found associated with p57 promoter within chromatin. All these observations suggest that, although different DNMTs may exert distinctive functions in p57 regulation, their effects are also influenced by the developmental phase and/or by the cell context in which they act. Moreover, it is possible to speculate that, once the imprinting has been established through the differential methylation of KvDMR1, the methylation status of the promoter becomes the main factor in determining the levels of p57 transcription. In line with a view in which the dominant outcome of reducing DNA methylation is the upregulation of the gene, treatment with DNA demethylating agents generally increases p57 expression, both from the paternal imprinted allele in mouse fibroblasts (121) and from the silenced gene in tumor cells (see below).

6.2. Histone modifications

Among the histone modifications associated with p57 regulation (Figure 2B), the acetylation of core histone tails is the most widely investigated. Histone acetylation is well recognized to affect gene expression both by directly relaxing the chromatin structure and by providing docking sites for bromodomain-containing proteins, thus facilitating the access of transcription factors (122). The status of H3 and H4 histone acetylation/deacetylation strongly correlates with p57 expression/repression in different regulatory contexts, such as imprinting, differentiation and tumorigenesis.

Regarding the imprinting regulation, studies focused on p57 promoter and gene body reported
Transcriptional regulation of \textit{p57}\textsuperscript{kip2} expression

lower levels of histone H3 lysine 9 and 14 acetylation (H3K9/K14ac), as well as H3 lysine 4 dimethylation (H3K4me2), another active histone mark, on the paternal respect to the maternal allele after the establishment of imprinting (109, 110). In contrast, as also observed for DNA methylation, the allele-specific pattern of histone acetylation at KvDMR1 is the opposite, with hyperacetylation of the paternal and hypoacetylation of the maternal allele.

A correlation between \textit{p57} expression and histone H3K9/14 acetylation was also observed in differentiating neuronal cells, in which the upregulation of \textit{p57} is concomitant with the increased acetylation at the transcriptional start site and at the first exon of the gene (123). Similarly, a number of tumor cell lines in which \textit{p57} expression is silenced showed, in addition to DNA hypermethylation around the transcriptional start site, also a significant level of histone hypoacetylation at the same region, when compared to tumor cell lines in which \textit{p57} is expressed (77, 124). Consistently with a critical role of histone acetylation in \textit{p57} regulation, the histone deacetylases HDAC1 and HDAC2 directly interact with \textit{p57} promoter. Moreover, their depletion in mouse embryo fibroblasts and in B cells causes cell cycle arrest accompanied by \textit{p57} upregulation (125).

Among the large number of other possible histone modifications, the trimethylation of lysine 27 of histone H3 (H3K27me3), a typical mark of facultative heterochromatin (126), also plays a recognized role in \textit{p57} regulation (Figure 2B). In cancer cells in which \textit{p57} is not expressed, \textit{p57} promoter, and even more the region downstream of the TSS, are enriched of both H3K27me3 and EZH2, the enzymatic subunit of the Polycomb repressive complex 2 (PRC2) responsible for the deposition of this mark, (127). This study also showed that the reduction of H3K27me3 at the \textit{p57} locus, caused by the depletion or the inhibition of EZH2, reactivates \textit{p57} expression, thus supporting the role of this modification in \textit{p57} silencing. Similarly, EZH2 and H3K27me3 have been shown to be required for the repression of \textit{p57} promoter in mature glial cells, in which this CK1, unlike in other cell systems, functions as an inhibitor of Schwann cell maturation (128). Finally, H3K27me3 has been reported as one of the repressive chromatin marks present on the paternal \textit{p57} allele (109, 110).

In line with the requirement of an inactive imprinting control region for allowing maternal \textit{p57} expression, H3K27me3 as well as the di- and trimethylation of lysine 9 of the histone H3 (H3K9me2/3), another mark of facultative heterochromatin (126), are also present on the maternal but not on the paternal KvDMR1 allele (110). However, it is worth recalling that KvDMR1 is a functionally complex region and that, as mentioned above, it is also bound by transcription factors, such as MyoD, that positively regulate \textit{p57} expression. In this regard we found that an aberrant enrichment of H3K9me2 at maternal KvDMR1 correlates with the unresponsiveness of \textit{p57} to the MyoD-dependent induction in some myogenic cell types. This is associated with the inability of MyoD to bind to KvDMR1, probably due to reduced chromatin accessibility of the binding region (100). This finding indicates that the same type of epigenetic modification at KvDMR1 can mediate both positive and negative regulation of \textit{p57} expression, depending on the concerted effects of additional regulatory factors. Moreover, it also calls attention to the epigenetic status of KvDMR1 as a critical determinant of the imprinting-independent silencing of \textit{p57}.

As assumed for a variety of genomic loci requiring a tight control of chromatin structure, the combination of multiple epigenetic modifications on the multiple \textit{p57} regulatory elements likely reflects the necessity of an accurate dosage of its expression and of a very specific, spatial and temporal expression pattern. While some information has been acquired on how the above modifications affect in general the accessibility to transcription, much less is known on how the modifications themselves are regulated. It is becoming increasingly evident the occurrence of a cross-talk between different histone modifications and between histone modifications and DNA methylation. This crosstalk, based on the recruitment or exclusion of specific chromatin-modifying enzymes by specific modified histone residues or methylated DNA, aims at reinforcing and maintaining certain epigenetic states. In contrast, much less is known about the molecular mechanisms by which specific chromatin modifications are initially deposited on specific regions. A commonly accepted view is that DNA-binding transcription factors recruit chromatin-modifying enzymes at their recognition sites but, more recently, an increasing interest is being focused on the role of non-coding RNAs in this process.

6.3. LncRNAs

Among the lncRNAs involved in \textit{p57} transcriptional regulation, the macro lncRNA \textit{Kcnq1ot1} (also called \textit{LIT1}) has emerged as a critical regulator of the chromatin status of the gene, at least in relation to the imprinting control. \textit{Kcnq1ot1} was first discovered, in both human and mouse, as a KvDMR1-associated RNA, expressed from the paternal allele and transcribed in antisense orientation to the maternally expressed \textit{Kcnq1} gene (129, 130). Its promoter was mapped to a KvDMR1sub-region, which is hypermethylated on the maternal and hypomethylated on the paternal allele, causing the imprinted expression of the gene (131, 132). \textit{Kcnq1ot1} is a ~ 90 kb RNA exclusively localized in the nucleus (133). Its role in the paternal imprinting was ascribed to its ability to spread \textit{in cis} along the domain and to direct chromatin modifications at the regulatory regions of the imprinted genes.
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(133–135). In particular, Kcnq1ot1 has been found associated with chromatin at specific regions of the imprinting domain, including p57 promoter (133, 135). Importantly, in placental tissue, Kcnq1ot1 interacts with members of histone methyltransferase complexes such as G9a, Suz12 and Ezh2 and its chromatin interactions at p57 and other imprinted loci correlate with the enrichment of the repressive histone modifications targeted by these enzymes, H3K9me3, H3K9me2 and H3K27me3 (109, 110, 133). Moreover, Kcnq1ot1 also interacts with Dnmt1 and this interaction is required for the Dnmt1 recruitment and for the maintenance of DNA hypermethylation at paternal p57 promoter (120, 136).

While the link between Kcnq1ot1 and p57 imprinting has been well established, nothing is yet known about the possibility that the lncRNA may also exert imprinting-independent functions in p57 regulation. However, a maternal Kcnq1ot1 transcript, starting from an alternative promoter and shorter than the paternal counterpart, has been recently detected in developing heart (137). Although this does not result in maternal p57 silencing, it cannot be excluded that maternal Kcnq1ot1 may play some role in modulating in cis the levels of maternal p57 transcription. It would be interesting to investigate whether maternal activation of Kcnq1ot1 and/or synthesis of functionally different Kcnq1ot1 RNA molecules also occur in other tissues and participate in the developmental regulation of p57.

Recently, it has emerged that other lncRNAs, such as Tug1 (138) and Linc00668 (139) in gastric cancer and HEIH (140) in hepatocellular carcinoma, recruit the PRC2 complex on p57 promoter. These findings suggest that different lncRNAs are involved in p57 regulation and that they act, at least to a certain extent, in a cell type-specific manner.

7. DEREGULATED p57 EXPRESSION AND DISEASE

The functional haploidy of p57, resulting from imprinting, makes it particularly vulnerable to genetic or epigenetic alterations leading to loss of function of the only active allele. In addition to inactivating point mutation in the p57 coding region, a large number of epigenetic changes causing p57 downregulation or silencing, have been associated to complex growth disorders. In general, the observed alterations seem to involve the inappropriate targeting to the maternal allele of epigenetic mechanisms normally employed for the transcriptional repression of the imprinted paternal allele. Consistently with the need of precisely controlled levels of p57, the failure of the paternal imprinting and the consequent biallelic expression of the gene, is also deleterious for the proper growth regulation.

As described below, the downregulation of p57 expression has been linked to cancer progression and to overgrowth diseases such as Beckwith-Wiedemann syndrome (BWS), as well as to focal hyperinsulinism and preeclampsia syndrome. Conversely, excess of p57 expression characterizes growth restriction syndromes such as Silver Russell (SRS), intra uterine growth restriction (IUGR) and IMAGE syndromes, and is associated with type 2-diabetes risk. In IMAGE syndrome, a very rare disease characterized by developmental defects and growth retardation, the phenotype is associated with an increased stability of the p57 protein rather than with a misregulation of its expression. We refer to another review addressing this issue (141).

7.1 Cancer

p57 alterations have been associated to human malignancies spanning from breast and liver tumors to hematological cancers (22, 24, 142). The expression levels of p57 increase at early stages of cancer progression and then decrease with the progression of the malignancy (143). This finding is in agreement with a recent study (67) that recognizes p57 as a target of the DNA damage response, a pathway often activated at the beginning of cancer progression (144). Since the loss of p57 function is associated with poor prognosis, the expression levels of this CKI have been proposed as a prognostic marker. Remarkably, whereas coding mutations in p57 have never been found in human cancers, the downregulation of gene expression has been frequently reported, highlighting not only the importance but also the fragility of the proper control of p57 expression. Both transcriptional and post-transcriptional mechanisms are involved in p57 downregulation in cancer cells. Concerning the latter, increasing evidence points to the over-expression of specific microRNAs as a frequent mechanism causing the reduction of p57 protein levels. We send the reader to other references on this subject (22, 145–147).

Concerning the molecular mechanisms leading to p57 transcriptional silencing, alterations of signaling pathways or their effectors and, above all, modifications of the chromatin status of either p57 promoter or KVDMR1 have been detected in cancer cells (Table 2). The epigenetic alterations of p57 cis-regulatory elements have attracted much interest of the scientific community because they can be manipulated and potentially reverted by epigenetic drugs, thus opening the way for the therapeutic re-expression of p57 (148).

7.1.1. Alterations of signaling pathways

Dysfunctions of signaling pathways appear to be responsible for p57 downregulation in some tumors. For example, accumulation of β-catenin, a downstream effector of Wnt signaling, is the most frequent molecular alteration in human adrenocortical cancer cells in which p57 is not expressed. Downregulation of β-catenin restores p57 expression...
Consistently with a direct role of DNA hypermethylation in p57 silencing, treatment with the DNA demethylating agents 5-Azacytidine or 5-aza-2'-deoxycytidine has been successfully used to restore p57 expression in several tumor cell types derived from leukemias and lymphomas (151, 152, 154), pancreatic (156), gastric (155), colorectal (124), lung (153) and breast cancers (100, 127).

Changes in the pattern of histone modifications at p57 promoter have also been involved in p57 silencing during tumorigenesis. Treatment with histone deacetylase (HDAC) inhibitors has been widely used to investigate the role of histone acetylation in regulating p57 expression. HDAC inhibitors are able to restore p57 expression in a high number of different cancer cell types, in some cases acting in synergy with DNA demethylating agents (124, 155–158). The de-repression of p57 expression was ascribed to the increased acetylation of p57 promoter, which allows the recruitment of Sp1 transcription factor (77). Moreover, in estrogen-responsive breast cancer cell lines, the inhibition of and leads to decreased proliferation and apoptosis of adrenocortical cancer cell lines, probably through the upregulation of p57 and of other members of Cip/Kip family (56). Moreover, it has been found that the downregulation of the p57 repressor Hes1 is able to increase p57 expression and to induce senescence in hepatocellular carcinoma cells (48). Finally, defective TGF β signaling has been associated to the failure to induce p57 expression and growth arrest in multiple myeloma cells (149).

### 7.1.2. Alterations of DNA methylation and histone modifications at p57 promoter

There are many evidences that DNA methylation is involved in p57 silencing during tumorigenesis, as suggested by the study of a wide range of cancer samples and cell lines (124, 150–155). Extensive analysis of the DNA methylation status of the p57 gene in several cancer cell lines and primary tumors pointed out that the region around the transcriptional start site is generally hypermethylated in tumor samples while it is barely methylated in normal tissues (124).

Consistently with a direct role of DNA hypermethylation in p57 silencing, treatment with the DNA demethylating agents 5-Azacytidine or 5-aza-2'-deoxycytidine has been successfully used to restore p57 expression in several tumor cell types derived from leukemias and lymphomas (151, 152, 154), pancreatic (156), gastric (155), colorectal (124), lung (153) and breast cancers (100, 127).

Changes in the pattern of histone modifications at p57 promoter have also been involved in p57 silencing during tumorigenesis.

<table>
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<tr>
<th>Regulator region</th>
<th>Epigenetic alteration</th>
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<td>Decreased histone acetylation</td>
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<td>Decreased H3K9me2</td>
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<td>Increased H3K9me2</td>
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Transcriptional regulation of p57<sup>ko</sup> expression

p57 expression by estrogen receptor α (ERα) has been associated to an increase of the repressive histone marks H3K27me3 and H3K9me2 at p57 promoter and gene body (159).

It has been reported that p57 promoter is regulated by at least two chromatin remodeling complexes found deregulated in cancer: SMARCB1 and NAP1L1. SMARCB1 is a component of SWI/SNF chromatin remodeling complexes and is frequently mutated or deleted in rhabdoid tumors. It has been demonstrated that SMARCB1 induces p57 expression by increasing H3 and H4 acetylation at p57 promoter (157). NAP1L1, a nucleosome assembly and histone exchange protein, has been shown to be involved in p57 repression in pancreatic neuroendocrine neoplasms by directly binding p57 promoter and by increasing promoter DNA methylation (160).

7.1.3. Alterations of DNA methylation and histone modifications at KvDMR1

As mentioned above, the epigenetic status of KvDMR1 generally influences p57 expression in an opposite manner respect to the epigenetic status of the promoter. Accordingly, loss of maternal KvDMR1 methylation is common to a variety of adult neoplasms, (161–164). A study in estrogen responsive breast cancer cell lines suggested that the ERα-dependent repression of p57 is associated not only to changes in histone methylation at the promoter but also to KvDMR1 hypomethylation, induction of Kcnq1ot1 expression and increase of CTCF binding to both KvDMR1 and p57 promoter (159). These alterations render the maternal KvDMR1 similar to the paternal one in its ability to silence p57. A study in esophageal cancer cell lines showed that KvDMR1, in addition to DNA hypomethylation, also presents the loss of the repressive histone mark H3K9me2 (165). However, we found that in breast cancer cell lines, lower levels of H3K9me2 at KvDMR1 correlate with higher levels of p57 expression (100). This finding is not in contrast with the previous ones, but probably reflects the complexity of KvDMR1, composed of multiple and functionally independent regulatory sub-regions, which can positively or negatively affect p57 expression. Consistently with this interpretation, the human KvDMR1 sub-region analyzed in our study displays a genomic organization, including E-boxes flanked by CTCF binding sites, similar to the homologous mouse sub-region bound by MyoD in muscle cells.

7.2 Overgrowth and growth restriction diseases

BWS has an incidence of 1:13700 and is characterized by a complex phenotype that includes malformation of several organs and a 1000-fold increased risk to develop embryonal tumors such as Wilm’s tumor, hepatoblastoma and rhabdomyosarcoma (91, 92). The clinical signature of BWS highlights the importance of p57 both in development and in cancer progression. Interestingly, embryos from mice knockout for p57 present many features of BWS patients (18) clearly indicating that p57 loss plays a central role in BWS.

Although mutations in the Igf2-H19 imprinting control region have been reported for around 10–20% of BWS patients, alterations in the epigenetic status of the p57 locus account for at least 60% of patients. In particular, about 50% of sporadic BWS cases present a loss of methylation at KvDMR1, which strongly correlates with p57 downregulation (166). On the other hand, in other BWS patients p57 downregulation is not associated with KvDMR1 hypomethylation. In those cases, depletion of the active mark histone H3K4me2 and enrichment of the repressive mark H3K9me2 were observed at the promoter level (167). These findings confirm that both the hypomethylation of KvDMR1 and the establishment of a repressive chromatin status at p57 promoter can contribute to p57 repression in BWS, just like in cancer cells. Interestingly, it has been reported that deletion of the maternal KvDMR1 leads to the expression of a truncated form of maternal Kcnq1ot1 and, consequently, to p57 downregulation and BWS phenotype (168). It has also been suggested that p57 downregulation in BWS can be due to a deletion in the second exon of Kcnq1 (169). Interestingly, the region involved is adjacent the most distal of the three putative p57 enhancers. Finally, 20% of patients present duplication or disomy of the silent paternal allele (170). The collection of alterations leading to p57 downregulation and to BWS phenotype confirms how promoter, enhancers, imprinting control region and maybe other still undescribed regulatory elements all contribute to p57 regulation.

SRS, a rare syndrome with an incidence of 1:100000, is characterized by severe intrauterine and postnatal growth retardation and is considered the clinical opposite of BWS (171). The genetic basis of SRS is heterogeneous, and it seems that the Igf2/H19 domain is more involved than the Kcnq1/Kcnq1ot1 domain in the onset of this pathology. However, several micro duplications of the 11p15 region, encompassing the genes Kcnq1, Kcnq1ot1 and p57, have been shown to lead to SRS if maternally inherited (172, 173). It is worth mentioning a study in mouse embryos, which took advantage of the expression of several BACs containing different combinations of the genes of the Kcnq1/Kcnq1ot1 domain, to suggest that p57 is the key gene of the domain to cause embryonic growth restriction (174). Although the direct involvement of p57 misregulation in SRS has not been completely demonstrated, it is conceivable to hypothesize that any alteration that leads to increased transcription of the gene may contribute to the etiology of the syndrome.
7.3. Diabetes and focal hyperinsulinism of infancy

p57 is expressed in pancreatic β-cells during development and in adult pancreas (175) and seems to play an important role in β-cell physiology, since its aberrant expression is associated to diabetes and focal hyperinsulinism of infancy.

A mouse model has been described in which a paternally inherited mutation in the second exon of the KCnq1 gene causes the downregulation of KCnq1ot1 and the upregulation of p57, accompanied by impaired β-cell proliferation, diminished insulin secretion and hyperglycemia (176). Consistently with a critical role of p57 in the observed phenotype, depletion of the CKI promotes replication of adult β-cells (177). However, the molecular mechanism leading to increased p57 transcription has not been investigated yet.

Further alterations within the KCnq1 locus have been associated with abnormal β-cell function. In particular, gene variants mapping in intron 10 (178), which includes the KvDMR1 region, and in intron 15 (179, 180), which is adjacent to the most proximal of the three putative p57 enhancers, correlate with type 2-diabetes risk. Although these studies did not address the possible involvement of p57 in the observed β-cell defects, it is attractive to hypothesize that the reported genetic changes may alter p57 expression.

Hyperinsulinism of infancy (HI) is a rare genetic disorder characterized by mild or severe hypoglycemia that can lead to irreversible neurological damage (181). The genetic basis of HI involves defects in genes that regulate insulin secretion from β-cells, such as ABCC8 and KCNJ11 encoding for an ATP-sensitive potassium channel. In the Focal HI, specific islets of β-cells have abnormal secretion of insulin and hyperplasia. In these cells, the mutation in the channel is accompanied by the loss of the maternal 11p15 region, which accounts for the overgrowth of β-cells (182). Indeed, it has been demonstrated that, while normal β-cells express p57 during different stages of development and in adult tissues, this expression is lost in focal HI (175). Moreover, the knockdown of p57 in adult β-cells promotes their proliferation, supporting a key role of p57 loss in the massive replication of β-cells that occurs in focal HI (177).

7.4. Placental diseases

In placenta, deficiency of p57 has been associated to pre-eclampsia while excess of p57 to intra-uterine growth restriction (IUGR). Pre-eclampsia is a hypertensive disorder of pregnancy, originating from abnormal placentation, which affects 6–10% of pregnancies, is one of the primary causes of premature birth and is associated with severe risks for both mother and fetus (183). Interestingly, pregnant mice carrying only the paternal silent allele of p57 showed symptoms recapitulating human pre-eclampsia (184). Correspondingly, it has been found that human placentas from women with pre-eclampsia presented low level of p57 (as well as of p27) compared to placentas from normal women (185).

On the other hand, IUGR is a multifactorial condition, with various causes, which leads to an insufficient growth of the fetus and early delivery. Three studies analyzed the expression levels of p57 and the methylation status of the locus in placentas from normal and IUGR patients and found that p57 is upregulated in this pathology (87, 186, 187). The analysis of the methylation status of p57 promoter, of KvDMR1 and of the putative enhancer regions showed contradictory results. In particular, while one study reported that the KvDMR1 region of IUGR placentas was hypomethylated compared to controls (186), the other two did not find any difference in the methylation status of the region. Remarkably, the putative p57 enhancer located at the position +97 kb was found hypermethylated in IUGR patients (87). All these findings indicate that the proper regulation of p57 transcription is critical for placental development and function.

8. CONCLUDING REMARKS

A unique role for p57 in mammalian development, differentiation and homeostasis was first suggested by the aberrant phenotype resulting from the gene knock-out in mice and by the correlation of a number of human diseases, ranging from growth disorders to cancer, with p57 misregulation. Subsequent studies, performed both in mouse and in human, revealed that the uniqueness of p57 lies not only in its ability to exert distinctive functions respect to the other Cip/Kip family members, but also in its highly specific and fine-tuned expression pattern.

Various signaling pathways, regulatory sequences, transcription factors and chromatin regulators have been involved in modulating p57 transcription in different cell types. In this regard, the skeletal muscle system, revealing the critical interplay between bHLH proteins, imprinting control regions, long-range chromatin interactions and epigenetic modifiers, offers a paradigmatic example of how multiple mechanisms cooperate in keeping p57 under precise control during developmental processes.

A more complete understanding of p57 transcriptional regulation and of its alterations in human diseases will require the discovery of additional regulatory sequences, the identification of further transcription factors acting in specific cell types relevant for the disease examined, and a better comprehension of the functions of co-factors, chromatin regulators and
IncRNAs. In light of the different, and in some cases opposite, impact that the same epigenetic change can have on p57 expression, depending on the regulatory region affected, particular effort should be made to get an integrated picture of the multiple p57 cis-acting elements and of the combinatorial effects of their epigenetic modifications.

These studies not only will provide a valuable model of the multiple and complex strategies devised by mammals for fine-tuning gene transcription during growth and differentiation, but also will have both diagnostic and therapeutic potential in the next future. The occurrence of mutations or epimutations in critical regulatory sequences, as well as the possible alterations of trans-acting factors interacting with these regions, could be used for the diagnosis of diseases associated with altered p57 expression. Moreover, the unravelling of the functions of epigenetic factors and of their mutual interplay promises the possibility of employing epigenetic drugs in order to restore the correct pattern of p57 expression.

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