Molecular dissection of Cdc6 and the miR-148 family : two stories with common themes

Duursma, A.M.

Chapter 1

Introduction
Molecular Mechanism of cancer development

Introduction

Molecular Mechanism of cancer development

Cancer is the result of a multi-step process in which cells acquire features that enable them to divide uncontrollably and to metastasize. Crucial steps in transformation of normal cells into malignant cells are the ability of cells to be self-sufficient in growth signals and to be insensitive to growth-inhibitory signals (Hanahan and Weinberg, 2000). As a consequence the cell cycle will be deregulated in favour of continues growth. The cell cycle is the period from one cell division to the next and can be divided into four phases. In G1, the first phase, mitogenic stimulation results in activation of cell cycle dependent kinases like Cyclin D1/CDK4 and Cylin E/ CDK2, which activate proteins involved in DNA replication and inhibit proteins that retain cells in a non-dividing state (Dulic et al., 1992; Koff et al., 1992; Morgan, 1997; Quelle et al., 1993). Cells that are not stimulated to divide in G1 enter the G0 state and can remain quiescent for longer periods of time. However, activated cells will enter the second phase, S-phase, in which DNA is duplicated and here for the activity of cyclin A/CDK2 is required (Rosenblatt et al., 1992). A schematic representation of the cell cycle and in which stages these different CDK/ Cyclin complexes are active is depicted in Figure 1. In G2-phase cells ensure that the DNA is properly replicated and that the conditions are right for the final separation of sister chromatids and cytokinesis in M-phase or mitosis (Smits and Medema, 2001).

In cancer the transition from G1-phase to S-phase is often deregulated due to altered gene function. Continues growth signalling can be a consequence of mutations in extracellular receptors or intracellular signal transducers, like the EGF receptor and Ras (Riese et al., 2007; Schubbert et al., 2007). However, it can also be due to amplifications of cell cycle activating proteins such as Cyclin D1 and cyclin E or loss of negative regulators of the cell cycle such as the CDK inhibitors p21 and p27 (Malumbres and Barbacid, 2001). Also loss of a functional Retinoblastoma (Rb) protein, which inhibits cell cycle progression by inhibiting the E2F transcription factor family that activate genes that are crucial for G1/S transition, is a frequent event in human cancers (Dannenberg and te Riele, 2006).

The basis of altered gene function often lies in genetic changes, which result in activation of oncogenes or repression of tumour suppressor genes. However, it has become increasingly clear that epigenetic abnormalities can contribute to tumourigenesis as well by altering patterns of gene expression (Jones and Baylin, 2007; Lund and van Lohuizen, 2004). Epigenetics can be described as the heritable changes in gene expression that are not accompanied by changes in DNA sequence. These include covalent modification of DNA and histone proteins, such as methylation. Silencing modifications will result in recruitment of proteins, which change the chromatin structure in a densely packed transcriptional inactive state. On the other hand activating modifications will recruit proteins that open the chromatin to make it accessible for

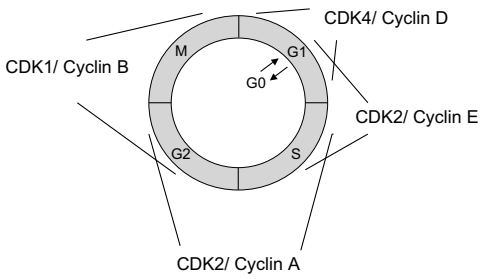

Figure 1. Schematic representation of CDK activity during different stages of the cell cycle. Lines show the overlapping activity ranges of different CDK/ Cyclin complexes.

the transcription machinery (Kouzarides, 2007). A marked example of epigenetic deregulation is the polycomb repressor gene BMI1, which overexpression in mouse cells has been shown to silence the INK4A locus that encodes for the tumour suppressor proteins p16 and p19ARF (Jacobs et al., 1999). Furthermore, BMI1 has been found overexpressed in several human tumours, suggesting that alterations in gene silencing indeed may contribute to tumourigenesis (Sparmann and van Lohuizen, 2006). The importance of epigenetic changes versus DNA mutations in cancer development was further underscribed by studies showing hypermethylation and thereby repression of promoter regions of tumour suppressor genes like Rb, VHL (von Hippel-Lindau), MLH1 and p16 (Gal-Yam et al., 2007). Based on these findings it has been proposed that promoter methylation can act in a similar way to gene mutation in cancer development.

DNA damage responses

Every cell division has a potential risk of creating a mistake in the genetic code. As a consequence the risk of developing cancer will increase with age (Serrano and Blasco, 2007). Moreover, the chance of genetic mutations is increased by exposure to DNA damage inducing agents such as UV light. Cells have evolved several mechanisms to protect themselves from DNA damage induced genetic abnormalities. Proteins like ATM and ATR will sense DNA damage and activate a genotoxic stress checkpoint that induces a cell cycle arrest or activation of repair proteins (Bartek and Lukas, 2007). Unrestorable damage will result in a permanent cell cycle arrest or apoptosis, depending on the cell type.

A central player in the DNA damage response is the transcription factor p53. In response to double strand breaks ATM will activate the CHK2 kinase (Matsuoka et al., 1998) that in turn will phosphorylate the p53 N-terminus (Chehab et al., 2000; Hirao et al., 2000; Shieh et al., 2000). This phosphorylation interferes with p53 binding to MDM2 (Chehab et al., 1999; Shieh et al., 1997; Siliciano et al., 1997). Significantly, MDM2 functions both in inhibiting p53 transcriptional activity and as a p53 E3-ligase (Michael and Oren, 2003). Therefore, phosphorylation of p53 in response to DNA damage will allow for its stabilization and activation. The key role of p53 in a DNA damage induced G1 arrest is mediated through its transcriptional target gene $p21^{Cip1}$ (el-Deiry et al., 1993),a cell cycle inhibitor that exerts its function by inhibiting CDK-cyclin complexes (Harper et al., 1993). Additionally, p53 can induce apoptosis by inducing transcription of proteins of the apoptotic machinery, such as Bax and Puma (Miyashita and Reed, 1995; Nakano and Vousden, 2001). Further, degradation of crucial cell cycle proteins can contribute to a G1 cell cycle arrest, such as the increased proteolysis of Cyclin D1, Cdc6 and Cdc25A (Agami and Bernards, 2000; Duursma and Agami, 2005; Mailand et al., 2000).

The importance of a proper DNA damage response is clear from a large number of heritable human diseases that arise from defects in checkpoints or DNA damage repair functions (Shiloh, 2003). Examples are the mutations in genes as ATM or BRCA1, which highly increase the risk of developing cancer. Patients with mutated ATM are diagnosed with the Ataxia telangiectasis syndrome and are immune deficient and are particularly predisposed to leukaemia's and lymphomas. Patients with a mutation in BRCA1 (Breast cancer 1) have an increased risk to develop breast cancer or ovarian cancer. Also in non-heritable forms of cancer genes participating in DNA damage responses are often lost. p53 is considered as one of the most important tumour suppressor proteins and its function is impaired in a large percentage of human tumours (Levine, 1997).

DNA replication

In S-phase, DNA is replicated in a tightly regulated manner to ensure proper duplication of the genome. In G1-phase of the cell cycle DNA replication is initiated at origins of replication, by binding of Cdc6 to the Origin Recognition Complex (ORC) (Liang et al., 1995) (Figure 2). This allows binding of Cdt1, which is a loading factor for the minichromosome maintenance (MCM)

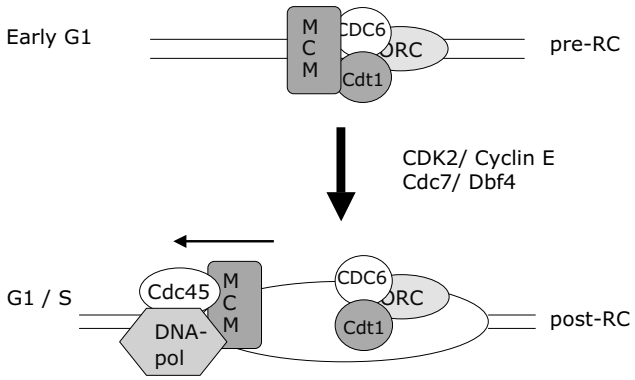

Figure 2. Schematic representation of initiation of DNA replication. CDK2/ Cyclin E as well as Cdc7/ Dbf4 activity convert a pre-RC (pre-Replication Complex) to a post-RC.

complex (Maiorano et al., 2000), a putative replicative helicase (Blow and Dutta, 2005). This prereplication complex (preRC) is triggered for replication at the onset of S-phase by binding of MCM10 (Wohlschlegel et al., 2002) and phosphorylation of preRC proteins by CDK2/Cyclin E and Cdc7/Dbf4. This facilitates the loading of Cdc45 and DNA polymerase-alpha (Arias and Walter, 2007). The activation of pre-RCs does not only account for accurate timing of replication, but also converts pre-RCs into post-RCs, which are not competent for initiation of replication.

The binding of Cdc6 and Cdt1 to the ORC complex in G1-phase is thought to be the limiting step in initiation of DNA replication and is therefore called replication licensing. To ascertain proper timing of origin licensing, both Cdt1 and Cdc6 are regulated by multiple pathways. Since both genes are targets of transcription factors of the E2F family, their expression increases in G1 when E2Fs are active (Hateboer et al., 1998; Yoshida and Inoue, 2004). To prevent re-licensing of replication origins Cdt1 is degraded in the beginning of S-phase by ubiquitin mediated proteolysis. In humans, two E3 ligases are involved in Cdt1 degradation, CUL4-DDB1CDT2 and SCFSkp2 (Li et al., 2003; Nishitani et al., 2006). In addition direct binding of Cdt1 to Geminin will inhibit its function (Wohlschlegel et al., 2000). Cdc6 protein stability has been shown to be regulated by the E3 ligase APC^{Cdh1} (Petersen et al., 2000). Interestingly, serine 54 phosphorylation of Cdc6 by CDK2/ cyclin E stabilizes the protein by interfering with APC^{Cdh1} mediated regulation (Duursma and Agami, 2005), which will be discussed in this thesis. Subsequently, it was shown by others that phosphorylation of this site by cyclin E plays a role in exit from quiescence and entrance into the cell cycle (Mailand and Diffley, 2005).

Cells have evolved several checkpoints that prevent DNA replication in case of DNA damage. One of these checkpoints inhibits origin firing by downregulating the levels of licensing proteins. Cdt1 has been described to be degraded in response to gamma-irradiation in an ATM independent manner by the Cul4-Roc1 E3 ligase (Higa et al., 2003) or in an ATM and ATR dependent manner upon treatment with both UV and gamma-irradiation by the SCF^{Skp2} E3-ligase (Kondo et al., 2004). We found that also the licensing protein Cdc6 is degraded upon gamma-irradiation in a p53-dependent manner (Duursma and Agami, 2005) (described in this thesis). It has been reported by others that Cdc6 can also be degraded in a p53 independent manner, possibly by the Huwe1 E3 ligase (Blanchard et al., 2002; Hall et al., 2007). This indicates that following DNA damage both Cdt1 and Cdc6

protein abundance are regulated by multiple pathways as well, perhaps signifying the prevention of origin licensing in cells with damaged DNA.
Deregulation of initiation of DNA replication can be linked to cancer in several ways. It was shown that rereplication of the genome can be induced by deregulation of licensing factors. Overexpression of Cdt1 and Cdc6 along with Cyclin A resulted in rereplication of human cancer cells with inactive p53 (Vaziri et al., 2003). Further, loss of Geminin was shown to induce rereplication in the presence of functional p53 (Melixetian et al., 2004).
Interestingly, a role for aberrant DNA replication was suggested in oncogene induced senescence (Bartkova et al., 2006; Di Micco et al., 2006). Oncogene activation, such as expression of active RasV12, resulted in augmented numbers of active replication origins and changes in replication fork progression (Di Micco et al., 2006). This in turn resulted in a partly replicated and rereplicated genome, which induced senescence via activation of the DNA damage checkpoint. Abrogation of this checkpoint could prevent oncogene-induced senescence and resulted in tumour growth in a mouse model (Di Micco et al., 2006).

Epigenetics
In mammals, epigenetic gene silencing can be regulated by direct modifications of DNA, but also by modifications of histones, protein complexes around which the DNA is enfolded. DNA can be modified by DNA methyltransferases (DNMTs), which transfer methyl groups from a S-adenosyl methionine (SAM) methyl donor to cytosine residues in DNA at CpG dinucleotides. These CpG dinucleotides are underrepresented in the genome apart from discrete regions with high CpG content that are called CpG islands and these occur mostly in gene promoter regions and in repetitive DNA sequences (Gal-Yam et al., 2007). Histones can be modified at many sites by many different proteins, but two important families of proteins are the Polycomb group (PcG) proteins and the Thritorax Group (TrxG). The PcG proteins are assembled in complexes that can initiate repression by methylation of lysines such as histone H3 lysine 27 (H3K27) in histone tails, and complexes that maintain silencing by facilitating additional remodelling of the chromatin (Schuettengruber et al., 2007). The activity of silencing complexes can be counteracted by complexes that promote transcriptionally active chromatin. During development the Thritorax Group (TrxG) antagonizes the effects of PcG group proteins by methylating histone H3K4 and thereby marking the chromatin as active (Schuettengruber et al., 2007). Mixed Lineage Leukemia 1 (MLL1) is a human TrxG homologue and a novel regulatory mechanism of this gene will be discussed in chapter 5.

Genomic DNA methylation of cytosines can be established by the 'de novo' methyltransferases Dnmt3a and Dnmt3b, which are able to methylate unmethylated DNA during early development and gametogenesis (Okano et al., 1999). This genomic imprinting coincides with their high expression in embryonic stem cells, whereas Dnmt3a and Dnmt3b are expressed to a low extent in differentiated somatic cells (Okano et al., 1998). On the other hand Dnmt1, the maintenance DNA methyltransferase, is merely expressed in somatic cells. It has a strong preference for hemi-methylated DNA and was shown to be the factor that preserves methylation marks on newly synthesized DNA during DNA replication (Gruenbaum et al., 1982; Leonhardt et al., 1992). Inactivation of a single Dnmt in mice results in embryonic lethality or the mice die shortly after birth, indicating that all three methyltransferases are essential for normal development (Li et al., 1992; Okano et al., 1999).
Although Dnmt3a and Dnmt3b have overlapping functions in imprinting of genes, Dnmt3b was shown to be essential for methylation of centromeric minor satellite repeats (Okano et al., 1999; Xu et al., 1999). Furthermore, inactivating mutations in the human Dnmt3b gene were linked to the Immunodeficiency, Centromeric

region instability and Facial anomalies (ICF) syndrome (Xu et al., 1999) and in all tissues of these patients reduced methylation of the minor satellite repeats was observed (Jeanpierre et al., 1993). Interestingly, lymphocytes of these patients show a high frequency of chromosomal abnormalities of chromosome 1, 9 and 16 such as centromeric decondensation and chromosome and chromatid breaks that are mostly restricted to the juxtacentromeric regions. In addition, multiradiate structures of chromosomes are observed (Maraschio et al., 1988; Sumner et al., 1998; Tuck-Muller et al., 2000). ICF patients are also characterized by low numbers of T-cells and this was recapitulated in a mouse model for the ICF syndrome. In this model an ICF-like mutation in Dnmt3b was shown to result in decreased T-cell survival (Ueda et al., 2006). From a study with conditional Dnmt3b knock-out mice it appeared that Dnmt3b is not involved in lymphoid lineage differentiation, but it does play a critical role in hematopoietic stem cell renewal (Tadokoro et al., 2007).

Initially, aberrant DNA hypomethylation was proposed to play a role in cancer, since tumour cells were shown to have a reduced content of methylated cytosines and a reduced amount of methylated genes (Feinberg and Vogelstein, 1983; Gama-Sosa et al., 1983). This hypomethylation was shown to result in reactivation of imprinted oncogenes, which in the case of Insulin Growth Factor 2 (IGF2) was associated with an increased risk for colon cancer (Cui et al., 2003; Sakatani et al., 2005). In addition, DNA hypomethylation was shown to promote chromosomal instability and it was shown to be sufficient to induce T-cell lymphomas in a mouse model (Eden et al., 2003; Gaudet et al., 2003).
In contrast, DNA hypermethylation of promoter regions was shown to promote tumourigenesis by repressing tumour suppressor gene activity. CpG islands in the promoter of the Rb tumour suppressor gene were the first discovered aberrantly methylated sequences in cancer (Greger et al., 1989). This was followed by several studies showing that promoter hypermethylation correlated with reduced expression, such as methylation of the p16 gene in bladder cancer (Gonzalez-Zulueta et al., 1995) and the mismatch repair gene hMLH1 in colon cancer (Kane et al., 1997). From the above it is clear that a tight control of DNA methyltransferase abundance is required. In chapter 4 of this thesis we describe a novel regulatory mechanism of Dnmt3b by a miRNA family.

MicroRNAs
MicroRNAs (miRNAs) are endogenous non-coding single-stranded RNAs of about 19-25 nucleotides, which function as negative regulators of protein coding mRNA sequences (Bushati and Cohen, 2007). According to computational studies each miRNA can regulate hundreds of mRNA targets and more than 30% of animal genes may be miRNA targets (Brennecke et al., 2005; Krek et al., 2005; Lewis et al., 2005; Xie et al., 2005). Therefore it is not surprising that miRNA have been implicated in regulation of many biological processes, such as differentiation, apoptosis and metabolism (Bushati and Cohen, 2007).
miRNAs are generally transcribed by RNA polymerase II into large pri-miRNA transcripts (Cai et al., 2004; Lee et al., 2004), which are processed by the RNase III enzyme Drosha and it's co-factor Pasha into a approximately 70 nucleotide pre-miRNA that is folded in the characteristic stem-loop structure (Lee et al., 2003)(Figure 3). This pre-miRNA will be exported to the cytosol by RAN-GTP and exportin 5 (Lund et al., 2004; Yi et al., 2003), where it will be processed further by Dicer, another RNase III enzyme (Hutvagner et al., 2001). Dicer cuts the loop of pre-miRNAs to generate a double stranded mature miRNA of which one strand will be loaded onto the miRNA-associated multiprotein RNA-induced silencing complex (miRISC), which directs the miRNA to its target mRNA (Carmell and Hannon, 2004). Several studies indicate that nucleotides 2-8 of the 5' end of the mature miRNA (the miRNA seed) are important in mRNA target recognition (Doench and Sharp, 2004; Lewis

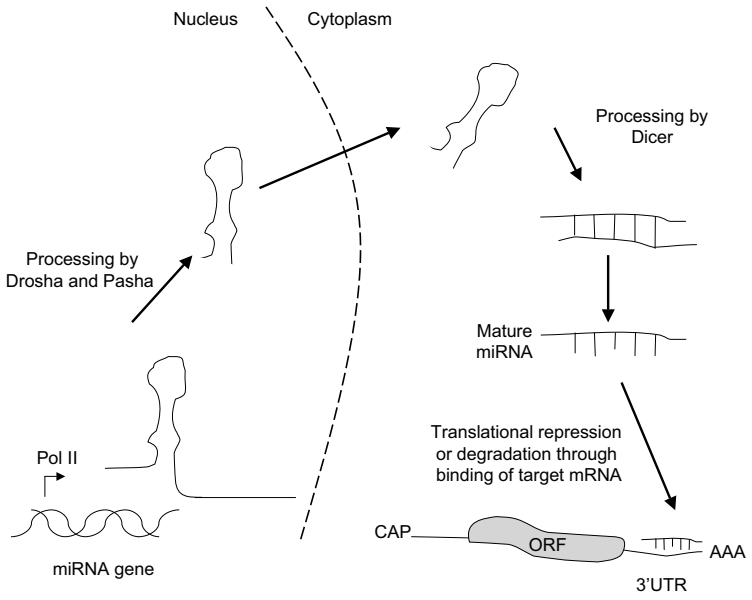

Figure 3. Schematic representation of miRNA biogenesis.

et al., 2005). In animals, miRNAs exert their function by binding with imperfect complementarity to the 3'UTR of protein coding mRNA sequences. This results in translational repression and enhanced RNA decay, possibly through reduced adenylation of the mRNA (Standart and Jackson, 2007). However, it has also been described that in case of near-perfect homology of the miRNA with the 3'UTR, the target mRNA can be cleaved in manner similar to siRNA-guided cleavage (Yekta et al., 2004). Also in plants miRNAs bind target mRNA with very high sequence complementarity and can induce both translational repression or siRNA-like RNA cleavage (Chen, 2004; Llave et al., 2002; Rhoades et al., 2002). However, in contrast to mammalian miRNAs, which target 3'UTRs, most plant miRNAs target protein coding sequences (CDS). Currently, no functional miRNA binding sites have been identified in mammalian CDS, nevertheless, computational approaches predict that CDS miRNA targets are present in mammals as well (Miranda et al., 2006). In this thesis we show for the first time an example of a human miRNA that regulates the protein abundance of Dnmt3b by binding to its coding region with high sequence complementarity.

With the possibility of miRNAs to regulate key cellular processes like cell growth and apoptosis, impaired miRNA expression has been implicated in tumourigenesis. Like protein encoding genes, deregulation of miRNA encoding genes can occur through genetic alterations such as amplifications, deletions and point mutations. One of the first examples was the frequent deletion of miR-15a and miR-16-1 in chronic lymphocytic leukemia (CLL) (Calin et al., 2002), which where shown later to negatively regulate the anti-apoptotic oncogene BCL2 (Cimmino et al., 2005). Also certain transcripts of the let-7 miRNA family were shown to be down-regulated in human lung cancer and this correlated with decreased post-operative survival (Takamizawa et al., 2004). This suggested a role for let-7 as a tumour suppressor and this was further supported by evidence that the let-7 family can negatively regulate Ras (Johnson et al.,

2005). In addition, it has been shown that let-7 can regulate the HMGA2 oncogene (Lee and Dutta, 2007; Mayr et al., 2007). Notably, HMGA2 translocates in certain leukaemia's thereby losing its 3'UTR with let-7 target sites, which renders the HMGA2 fusion protein insensitive to let-7 regulation.
Conversely, the expression of other miRNAs was found increased in tumours, such as the miR-17-92 cluster on chromosome 13 that was shown to be amplified in human B-cell lymphoma's (He et al., 2005). Remarkably, this cluster was shown to be upregulated by MYC and to modulate E2F1 expression (O'Donnell et al., 2005), but also to collaborate with MYC by accelerating tumourigenesis in a mouse B-cell lymphoma model (He et al., 2005). In addition, miR-372 and miR-373 have been identified from a genetic screen for miRNAs that cooperate with oncogenic RAS to transform primary human cells, thereby bypassing activation of the p53 pathway (Voorhoeve et al., 2006). Interestingly, these miRNAs were found to be expressed in most testicular germ cell tumours, which are wild-type for p53. This suggests a role for miR-372 and miR-373 in the development of these tumours. Finally, the p27 tumour suppressor was shown to be regulated by miR-221 and miR-222 (le Sage et al., 2007). High expression of these miRNAs was found to correlate inversely with reduced expression of p27 in human glioblastoma's and reducing the levels of miR-221 in these cells resulted in decreased proliferation.

From the above it is clear that genetic alterations of human miRNAs can play a key role in tumourigenesis. However, human miRNAs expression can also be altered by epigenetic changes such as aberrant methylation of CpG islands in promoter regions. Treatment of cancer cells with a demethylating agent resulted in increased expression of miR-127 and BCL6 was identified as a target (Saito et al., 2006). In another study CpG methylation status of miRNAs were analysed in breast tissues and here miRNA-9-1 was demonstrated to be methylated in breast tumour tissues but not in normal tissue (Lehmann et al., 2007).
Lastly, altered transcriptional activation of miRNAs might contribute to the process of tumourigenesis. This was discussed above for the miR-17-92 cluster, which is regulated by MYC. However, recent studies identified several miRNAs that are regulated by the tumour suppressor protein p53 of which miR-34 was most prominent (Bommer et al., 2007; Chang et al., 2007; Corney et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tarasov et al., 2007). Significantly, p53 was shown to directly target the miR-34 promoter region and loss of p53 resulted in decreased miR-34 expression. Ectopic expression of miR-34 induced apoptosis and a cell cycle arrest, thereby suppressing tumour cell proliferation. This indicates that reduced miRNA expression due to functional inactivation of well-known tumour suppressor pathways might contribute to the process of carcinogenesis as well.

Outline of this thesis

The studies described in this thesis aimed to gain more insight in the regulation and function of the Cdc6 and the miR-148/152 family.

In Chapter 2 we studied the effect of DNA damage on Cdc6 protein stability. We identified that phosphorylation of Cdc6 at Serine 54 by CDK2/ Cyclin E stabilises the protein during normal replication. In response to DNA damage this kinase complex is inhibited in a p53 and p21-dependent manner, resulting in decreased Cdc6 protein level. In Chapter 3, the implications of these findings will be discussed in more detail.
Next, we studied several aspects of the function of the conserved miR-148/152 miRNA family. In chapter 4, we describe our finding that miR-148 regulates Dnmt3b mRNA through interacting with its protein coding sequence. Notably, all current described mammalian miRNAs target mRNAs by binding to mRNA 3'UTRs. In Chapter 5, we extended this study by exploring whether miR-148 mediated regulation of Dnmt3b plays a role in early human thymic development. Interestingly, we found differential expression of miR-148 in plasmacytoid

dendritic cells (pDCs) and its precursor cell. Moreover, exogenous expression of miR-148 resulted in a dramatic increase of pDCs in an in vitro assay, suggesting that miR-148 expression interferes with normal differentiation, survival or apoptosis. In Chapter 6, we describe a role for miR-152 in S and G2/M-phase of cell cycle progression in diploid fibroblasts. These findings will be generally discussed in Chapter 7.

References

Agami, R., and Bernards, R. (2000). Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage. Cell 102, 55-66.

Arias, E. E., and Walter, J. C. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. Genes Dev 21, 497-518.

Bartek, J., and Lukas, J. (2007). DNA damage checkpoints: from initiation to recovery or adaptation. Curr Opin Cell Biol 19, 238-245.

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L. V., Kolettas, E., Niforou, K., Zoumpourlis, V. C., et al. (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 444, 633-637.

Blanchard, F., Rusiniak, M. E., Sharma, K., Sun, X., Todorov, I., Castellano, M. M., Gutierrez, C., Baumann, H., and Burhans, W. C. (2002). Targeted destruction of DNA replication protein Cdc6 by cell death pathways in mammals and yeast. Mol Biol Cell 13, 1536-1549.

Blow, J. J., and Dutta, A. (2005). Preventing re-replication of chromosomal DNA. Nat Rev Mol Cell Biol 6, 476-486.

Bommer, G. T., Gerin, I., Feng, Y., Kaczorowski, A. J., Kuick, R., Love, R. E., Zhai, Y., Giordano, T. J., Qin, Z. S., Moore, B. B., et al. (2007). p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol 17, 1298-1307.

Brennecke, J., Stark, A., Russell, R. B., and Cohen, S. M. (2005). Principles of microRNA-target recognition. PLoS Biol 3, e85.

Bushati, N., and Cohen, S. M. (2007). microRNA Functions. Annu Rev Cell Dev Biol 23, 175-205.

Cai, X., Hagedorn, C. H., and Cullen, B. R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. Rna 10, 1957-1966.

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. (2002). Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 99, 15524-15529.

Carmell, M. A., and Hannon, G. J. (2004). RNase III enzymes and the initiation of gene silencing. Nat Struct Mol Biol 11, 214-218.

Chang, T. C., Wentzel, E. A., Kent, O. A., Ramachandran, K., Mullendore, M., Lee, K. H., Feldmann, G., Yamakuchi, M., Ferlito, M., Lowenstein, C. J., et al. (2007). Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 26, 745-752.

Chehab, N. H., Malikzay, A., Appel, M., and Halazonetis, T. D. (2000). Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. Genes Dev 14, 278-288.

Chehab, N. H., Malikzay, A., Stavridi, E. S., and Halazonetis, T. D. (1999). Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. Proc Natl Acad Sci U S A 96, 13777-13782.

Chen, X. (2004). A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. Science 303, 2022-2025.

Cimmino, A., Calin, G. A., Fabbri, M., Iorio, M. V., Ferracin, M., Shimizu, M., Wojcik, S. E., Aqeilan, R. I., Zupo, S., Dono, M., et al. (2005). miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 102, 13944-13949.

Corney, D. C., Flesken-Nikitin, A., Godwin, A. K., Wang, W., and Nikitin, A. Y. (2007). MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res 67, 8433-8438.

Cui, H., Cruz-Correa, M., Giardiello, F. M., Hutcheon, D. F., Kafonek, D. R., Brandenburg, S., Wu, Y., He, X., Powe, N. R., and Feinberg, A. P. (2003). Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 299, 1753-1755.

Dannenberg, J. H., and te Riele, H. P. (2006). The retinoblastoma gene family in cell cycle regulation and suppression of tumorigenesis. Results Probl Cell Differ 42, 183-225.

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P. G., Bensimon, A., et al. (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 444, 638-642.

Doench, J. G., and Sharp, P. A. (2004). Specificity of microRNA target selection in translational repression. Genes Dev 18, 504-511.

Dulic, V., Lees, E., and Reed, S. I. (1992). Association of human cyclin E with a periodic G1-S phase protein kinase. Science 257, 1958-1961.

Duursma, A., and Agami, R. (2005). p53-Dependent regulation of Cdc6 protein stability controls cellular proliferation. Mol Cell Biol 25, 6937-6947.

Eden, A., Gaudet, F., Waghmare, A., and Jaenisch, R. (2003). Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300, 455.

el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. Cell 75, 817-825.

Feinberg, A. P., and Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301, 89-92.

Gal-Yam, E. N., Saito, Y., Egger, G., and Jones, P. A. (2007). Cancer Epigenetics: Modifications, Screening, and Therapy. Annu Rev Med.

Gama-Sosa, M. A., Slagel, V. A., Trewyn, R. W., Oxenhandler, R., Kuo, K. C., Gehrke, C. W., and Ehrlich, M. (1983). The 5-methylcytosine content of DNA from human tumors. Nucleic Acids Res 11, 6883-6894.

Gaudet, F., Hodgson, J. G., Eden, A., Jackson-Grusby, L., Dausman, J., Gray, J. W., Leonhardt, H., and Jaenisch, R. (2003). Induction of tumors in mice by genomic hypomethylation. Science 300, 489-492.

Gonzalez-Zulueta, M., Bender, C. M., Yang, A. S., Nguyen, T., Beart, R. W., Van Tornout, J. M., and Jones, P. A. (1995). Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. Cancer Res 55, 4531-4535.

Greger, V., Passarge, E., Hopping, W., Messmer, E., and Horsthemke, B. (1989). Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 83, 155-158.

Gruenbaum, Y., Cedar, H., and Razin, A. (1982). Substrate and sequence specificity of a eukaryotic DNA methylase. Nature 295, 620-622.

Hall, J. R., Kow, E., Nevis, K. R., Lu, C. K., Luce, K. S., Zhong, Q., and Cook, J. G. (2007). Cdc6 stability is regulated by the Huwe1 ubiquitin ligase after DNA damage. Mol Biol Cell 18, 3340-3350.

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. Cell 100, 57-70.

Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993). The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75, 805-816.

Hateboer, G., Wobst, A., Petersen, B. O., Le Cam, L., Vigo, E., Sardet, C., and Helin, K. (1998). Cell cycle-regulated expression of mammalian CDC6 is dependent on E2F. Mol Cell Biol 18, 6679-6697.

He, L., He, X., Lim, L. P., de Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., Ridzon, D., et al. (2007). A microRNA component of the p53 tumour suppressor network. Nature 447, 1130-1134.

He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S. W., Hannon, G. J., and Hammond, S. M. (2005). A microRNA polycistron as a potential human oncogene. Nature 435, 828-833.

Higa, L. A., Mihaylov, I. S., Banks, D. P., Zheng, J., and Zhang, H. (2003). Radiation-mediated proteolysis of CDT1

by CUL4-ROC1 and CSN complexes constitutes a new checkpoint. Nat Cell Biol 5, 1008-1015.

Hirao, A., Kong, Y. Y., Matsuoka, S., Wakeham, A., Ruland, J., Yoshida, H., Liu, D., Elledge, S. J., and Mak, T. W. (2000). DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science 287, 1824-1827.

Hutvagner, G., McLachlan, J., Pasquinelli, A. E., Balint, E., Tuschl, T., and Zamore, P. D. (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293, 834-838.

Jacobs, J. J., Kieboom, K., Marino, S., DePinho, R. A., and van Lohuizen, M. (1999). The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. Nature 397, 164-168.

Jeanpierre, M., Turleau, C., Aurias, A., Prieur, M., Ledeist, F., Fischer, A., and Viegas-Pequignot, E. (1993). An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. Hum Mol Genet 2, 731-735.

Johnson, S. M., Grosshans, H., Shingara, J., Byrom, M., Jarvis, R., Cheng, A., Labourier, E., Reinert, K. L., Brown, D., and Slack, F. J. (2005). RAS is regulated by the let-7 microRNA family. Cell 120, 635-647.

Jones, P. A., and Baylin, S. B. (2007). The epigenomics of cancer. Cell 128, 683-692.

Kane, M. F., Loda, M., Gaida, G. M., Lipman, J., Mishra, R., Goldman, H., Jessup, J. M., and Kolodner, R. (1997). Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res 57, 808-811.

Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franza, B. R., and Roberts, J. M. (1992). Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. Science 257, 1689-1694.

Kondo, T., Kobayashi, M., Tanaka, J., Yokoyama, A., Suzuki, S., Kato, N., Onozawa, M., Chiba, K., Hashino, S., Imamura, M., et al. (2004). Rapid degradation of Cdt1 upon UV-induced DNA damage is mediated by SCFSkp2 complex. J Biol Chem 279, 27315-27319.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell 128, 693-705.

Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da Piedade, I., Gunsalus, K. C., Stoffel, M., and Rajewsky, N. (2005). Combinatorial microRNA target predictions. Nat Genet 37, 495-500.

le Sage, C., Nagel, R., Egan, D. A., Schrier, M., Mesman, E., Mangiola, A., Anile, C., Maira, G., Mercatelli, N., Ciafre, S. A., et al. (2007). Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. Embo J 26, 3699-3708.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., and Kim, V. N. (2003). The nuclear RNase III Drosha initiates microRNA processing. Nature 425, 415-419.

Lee, Y., Kim, M., Han, J., Yeom, K. H., Lee, S., Baek, S. H., and Kim, V. N. (2004). MicroRNA genes are transcribed by RNA polymerase II. Embo J 23, 4051-4060.

Lee, Y. S., and Dutta, A. (2007). The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 21, 1025-1030.

Lehmann, U., Hasemeier, B., Christgen, M., Muller, M., Romermann, D., Langer, F., and Kreipe, H. (2007). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. J Pathol.

Leonhardt, H., Page, A. W., Weier, H. U., and Bestor, T. H. (1992). A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. Cell 71, 865-873.

Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. Cell 88, 323-331.

Lewis, B. P., Burge, C. B., and Bartel, D. P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15-20.

Li, E., Bestor, T. H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69, 915-926.

Li, X., Zhao, Q., Liao, R., Sun, P., and Wu, X. (2003). The SCF(Skp2) ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1

degradation. J Biol Chem 278, 30854-30858.

Liang, C., Weinreich, M., and Stillman, B. (1995). ORC and Cdc6p interact and determine the frequency of initiation of DNA replication in the genome. Cell 81, 667-676.

Llave, C., Xie, Z., Kasschau, K. D., and Carrington, J. C. (2002). Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science 297, 2053-2056.

Lund, A. H., and van Lohuizen, M. (2004). Epigenetics and cancer. Genes Dev 18, 2315-2335.

Lund, E., Guttinger, S., Calado, A., Dahlberg, J. E., and Kutay, U. (2004). Nuclear export of microRNA precursors. Science 303, 95-98.

Mailand, N., and Diffley, J. F. (2005). CDKs promote DNA replication origin licensing in human cells by protecting Cdc6 from APC/C-dependent proteolysis. Cell 122, 915-926.

Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Welcker, M., Bartek, J., and Lukas, J. (2000). Rapid destruction of human Cdc25A in response to DNA damage. Science 288, 1425-1429.

Maiorano, D., Moreau, J., and Mechali, M. (2000). XCDT1 is required for the assembly of pre-replicative complexes in Xenopus laevis. Nature 404, 622-625.

Malumbres, M., and Barbacid, M. (2001). To cycle or not to cycle: a critical decision in cancer. Nat Rev Cancer 1, 222-231.

Maraschio, P., Zuffardi, O., Dalla Fior, T., and Tiepolo, L. (1988). Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial anomalies: the ICF syndrome. J Med Genet 25, 173-180.

Matsuoka, S., Huang, M., and Elledge, S. J. (1998). Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282, 1893-1897.

Mayr, C., Hemann, M. T., and Bartel, D. P. (2007). Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 315, 1576-1579.

Melixetian, M., Ballabeni, A., Masiero, L., Gasparini, P., Zamponi, R., Bartek, J., Lukas, J., and Helin, K. (2004). Loss of Geminin induces rereplication in the presence of functional p53. J Cell Biol 165, 473-482.

Michael, D., and Oren, M. (2003). The p53-Mdm2 module and the ubiquitin system. Semin Cancer Biol 13, 49-58.

Miranda, K. C., Huynh, T., Tay, Y., Ang, Y. S., Tam, W. L., Thomson, A. M., Lim, B., and Rigoutsos, I. (2006). A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 126, 1203-1217.

Miyashita, T., and Reed, J. C. (1995). Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 80, 293-299.

Morgan, D. O. (1997). Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 13, 261-291.

Nakano, K., and Vousden, K. H. (2001). PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 7, 683-694.

Nishitani, H., Sugimoto, N., Roukos, V., Nakanishi, Y., Saijo, M., Obuse, C., Tsurimoto, T., Nakayama, K. I., Nakayama, K., Fujita, M., et al. (2006). Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. Embo J 25, 1126-1136.

O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V., and Mendell, J. T. (2005). c-Myc-regulated microRNAs modulate E2F1 expression. Nature 435, 839-843.

Okano, M., Bell, D. W., Haber, D. A., and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99, 247-257.

Okano, M., Xie, S., and Li, E. (1998). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 19, 219-220.

Petersen, B. O., Wagener, C., Marinoni, F., Kramer, E. R., Melixetian, M., Lazzerini Denchi, E., Gieffers, C., Matteucci, C., Peters, J. M., and Helin, K. (2000). Cell cycle- and cell growth-regulated proteolysis of mammalian CDC6 is dependent on APC-CDH1. Genes Dev 14, 2330-2343.

Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J. Y., Bar-Sagi, D., Roussel, M. F., and Sherr, C. J. (1993). Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. Genes Dev 7, 1559-1571.

Raver-Shapira, N., Marciano, E., Meiri, E., Spector, Y., Rosenfeld, N., Moskovits, N., Bentwich, Z., and Oren, M. (2007). Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 26, 731-743.

Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B., and Bartel, D. P. (2002). Prediction of plant microRNA targets. Cell 110, 513-520.

Riese, D. J., 2nd, Gallo, R. M., and Settleman, J. (2007). Mutational activation of ErbB family receptor tyrosine kinases: insights into mechanisms of signal transduction and tumorigenesis. Bioessays 29, 558-565.

Rosenblatt, J., Gu, Y., and Morgan, D. O. (1992). Human cyclin-dependent kinase 2 is activated during the S and G2 phases of the cell cycle and associates with cyclin A. Proc Natl Acad Sci U S A 89, 2824-2828.

Saito, Y., Liang, G., Egger, G., Friedman, J. M., Chuang, J. C., Coetzee, G. A., and Jones, P. A. (2006). Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 9, 435-443.

Sakatani, T., Kaneda, A., Iacobuzio-Donahue, C. A., Carter, M. G., de Boom Witzel, S., Okano, H., Ko, M. S., Ohlsson, R., Longo, D. L., and Feinberg, A. P. (2005). Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science 307, 1976-1978.

Schubbert, S., Shannon, K., and Bollag, G. (2007). Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 7, 295-308.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B., and Cavalli, G. (2007). Genome regulation by polycomb and trithorax proteins. Cell 128, 735-745.

Serrano, M., and Blasco, M. A. (2007). Cancer and ageing: convergent and divergent mechanisms. Nat Rev Mol Cell Biol 8, 715-722.

Shieh, S. Y., Ahn, J., Tamai, K., Taya, Y., and Prives, C. (2000). The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev 14, 289-300.

Shieh, S. Y., Ikeda, M., Taya, Y., and Prives, C. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91, 325-334.

Shiloh, Y. (2003). ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 3, 155-168.

Siliciano, J. D., Canman, C. E., Taya, Y., Sakaguchi, K., Appella, E., and Kastan, M. B. (1997). DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 11, 3471-3481.

Smits, V. A., and Medema, R. H. (2001). Checking out the G(2)/M transition. Biochim Biophys Acta 1519, 1-12.

Sparmann, A., and van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 6, 846-856.

Standart, N., and Jackson, R. J. (2007). MicroRNAs repress translation of m7Gppp-capped target mRNAs in vitro by inhibiting initiation and promoting deadenylation. Genes Dev 21, 1975-1982.

Sumner, A. T., Mitchell, A. R., and Ellis, P. M. (1998). A FISH study of chromosome fusion in the ICF syndrome: involvement of paracentric heterochromatin but not of the centromeres themselves. J Med Genet 35, 833-835.

Tadokoro, Y., Ema, H., Okano, M., Li, E., and Nakauchi, H. (2007). De novo DNA methyltransferase is essential for self-renewal, but not for differentiation, in hematopoietic stem cells. J Exp Med 204, 715-722.

Takamizawa, J., Konishi, H., Yanagisawa, K., Tomida, S., Osada, H., Endoh, H., Harano, T., Yatabe, Y., Nagino, M., Nimura, Y., et al. (2004). Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 64, 3753-3756.

Tarasov, V., Jung, P., Verdoodt, B., Lodygin, D., Epanchintsev, A., Menssen, A., Meister, G., and Hermeking, H. (2007). Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. Cell Cycle 6, 1586-1593.

Tuck-Muller, C. M., Narayan, A., Tsien, F., Smeets, D. F., Sawyer, J., Fiala, E. S., Sohn, O. S., and Ehrlich, M. (2000). DNA hypomethylation and unusual chromosome instability in cell lines from ICF syndrome patients. Cytogenet Cell Genet 89, 121-128.

Ueda, Y., Okano, M., Williams, C., Chen, T., Georgopoulos, K., and Li, E. (2006). Roles for Dnmt3b in mammalian development: a mouse model for the ICF syndrome. Development 133, 1183-1192.

Vaziri, C., Saxena, S., Jeon, Y., Lee, C., Murata, K., Machida, Y., Wagle, N., Hwang, D. S., and Dutta, A. (2003). A p53-dependent checkpoint pathway prevents rereplication. Mol Cell 11, 997-1008.

Voorhoeve, P. M., le Sage, C., Schrier, M., Gillis, A. J., Stoop, H., Nagel, R., Liu, Y. P., van Duijse, J., Drost, J., Griekspoor, A., et al. (2006). A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 124, 1169-1181.

Wohlschlegel, J. A., Dhar, S. K., Prokhorova, T. A., Dutta, A., and Walter, J. C. (2002). Xenopus Mcm10 binds to origins of DNA replication after Mcm2-7 and stimulates origin binding of Cdc45. Mol Cell 9, 233-240.

Wohlschlegel, J. A., Dwyer, B. T., Dhar, S. K., Cvetic, C., Walter, J. C., and Dutta, A. (2000). Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. Science 290, 2309-2312.

Xie, X., Lu, J., Kulbokas, E. J., Golub, T. R., Mootha, V., Lindblad-Toh, K., Lander, E. S., and Kellis, M. (2005). Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. Nature 434, 338-345.

Xu, G. L., Bestor, T. H., Bourc'his, D., Hsieh, C. L., Tommerup, N., Bugge, M., Hulten, M., Qu, X., Russo, J. J., and Viegas-Pequignot, E. (1999). Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402, 187-191.

Yekta, S., Shih, I. H., and Bartel, D. P. (2004). MicroRNA-directed cleavage of HOXB8 mRNA. Science 304, 594-596.

Yi, R., Qin, Y., Macara, I. G., and Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17, 3011-3016.

Yoshida, K., and Inoue, I. (2004). Regulation of Geminin and Cdt1 expression by E2F transcription factors. Oncogene 23, 3802-3812.