

## Gene Section

### Review

# CDKN2B (cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4))

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## Identity

**Other names:** CDK4I; INK4B; MTS2; P15; TP15; p15INK4b

**HGNC (Hugo):** CDKN2B

**Location:** 9p21.3

## DNA/RNA

### Description

The p15INK4B gene encompasses 6.41 Kb of DNA and has 2 coding exons. It is tandemly linked to p16INK4A and p14ARF within 42 Kb of genomic locus located on chromosome 9p21. The locus is commonly referred to as INK4/ARF locus.

### Transcription

CDKN2B gene encodes 2 distinct transcript variants: p15 and p10. p10 arises from an alternative 5' splice donor site within intron 1 of:

- p15.
- p15: 3.82 Kb of mRNA.
- p10: 0.86 Kb of mRNA.

Regulation: A conserved DNA element with the ability to regulate the entire INK4/ARF locus has been identified in close proximity of the locus and named regulatory domain (RD). It appears to promote transcriptional repression of all three genes encoded by the locus, in a manner dependent on CDC6. In proliferating embryonic fibroblasts (MEFs), EZH2 a

member of the polycomb repressive complex 2 (PRC2) as well as BMI1 and M33 members of the polycomb repressive complex 1 (PRC1) are strongly expressed and are found to localize to the INK4/ARF RD. BMI1 has been shown to interact specifically with CDC6. These polycomb group (PcG) complexes repress the locus activity through the establishment of repressive chromatin modifications such as H3K27 trimethylation. During senescence, binding of these complexes to RD is lost and correlates with increased expression of the INK4/ARF genes (Figure 1).

The p15INK4B gene is also silenced by a long non coding RNA, called antisense non-coding RNA in the INK4 locus (ANRIL), whose expression was found to be inversely related to the expression of p15INK4B in leukemia cell lines. It was shown that ANRIL induces the silencing of p15INK4B in cis and trans by triggering heterochromatin formation in a Dicer-independent manner. PcG complexes are recruited to the INK4/ARF locus by ANRIL and modulate its repression (Figure 1). Additionally, a naturally occurring antisense circular ANRIL RNAs (cANRIL) has also been described. Different forms of cANRIL are produced in most INK4/ARF expressing cells, suggesting that alternative splicing events leading to different ANRIL structures can contribute to changes in PcG-mediated INK4/ARF repression.

Specific transcription regulators of p15INK4B have also been reported (see Figure 2). These include TGF- $\beta$ , MIZ-1, SMAD3/SMAD4 complex, SP1, c-MYC, IRF8, PU.1, SNAIL and EGR1 factors among others.

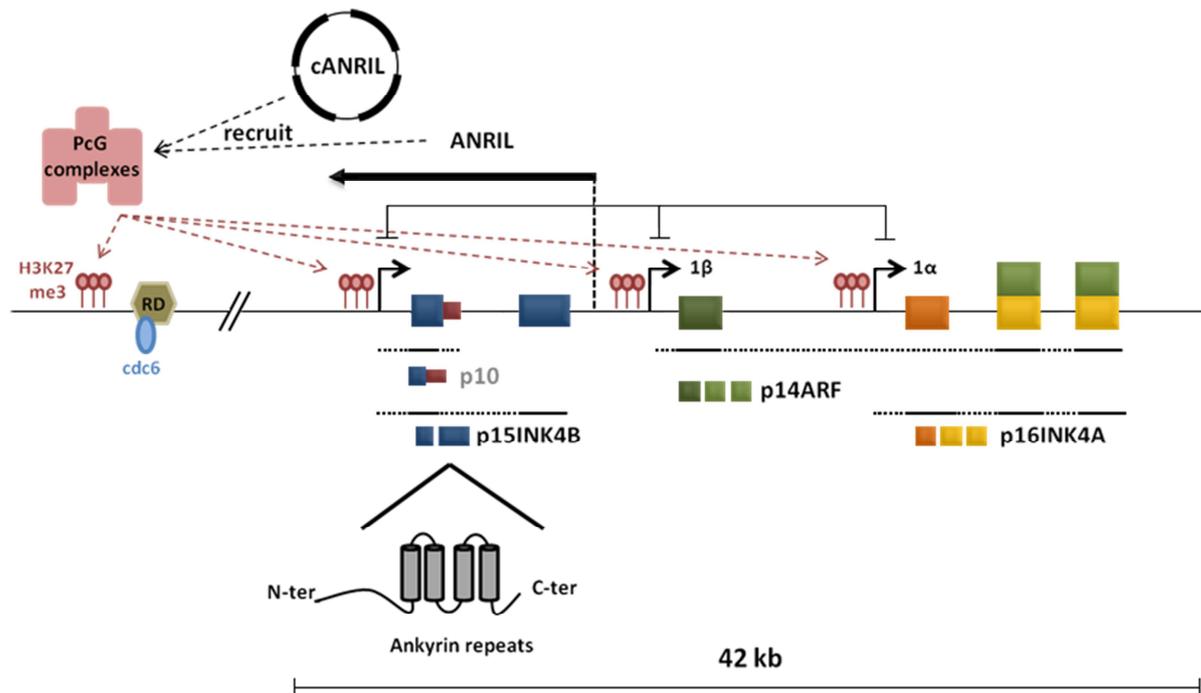


Figure 1.

p15INK4B expression is dramatically induced by TGF- $\beta$ , suggesting that it is a potent downstream effector of TGF- $\beta$  mediated growth arrest.

MIZ-1 is a transcription factor that has been shown to bind the initiator element (Inr) in the promoter region and induce the transcription of p15INK4B in epithelial cells. However it can also recruit transcriptional co-repressors such as c-MYC and GFI-1 to the promoter region by binding to them and forming inhibitory complexes. TGF- $\beta$  has been reported to re-activate the core promoter through downregulation of C-MYC and GFI-1, thereby releasing endogenous MIZ-1 from inhibition.

The SMAD3/4 complex readily forms following TGF- $\beta$  treatment and physical interaction between this complex, MIZ-1 and promoter-bound SP1 protein has been described. These interactions have been proposed to constitute a platform for the recruitment of co-activators, and do not seem to be affected by the suppressor activity of c-MYC. The inhibitory function of c-MYC seems to be cell-type dependent, as it was confirmed in epithelial cells but not in the hematopoietic lineage. In myeloid cells, the transcription factor c-MYB was shown to prevent the transcription and the upregulation of p15INK4B which is normally associated with the differentiation process. The mechanism by which C-MYB does this is unclear but it is not through upregulation of c-MYC, a known target of c-MYB.

A tri-component transcriptional complex consisting of

SNAIL, SP1 and EGR-1 was also described for its ability to trigger the p15INK4b promoter activation upon TPA treatment.

In murine myeloid cells specifically, the interferon consensus sequence-binding protein/interferon regulatory factor 8 (ICSBP/IRF-8) in combination with PU.1 were shown to bind p15Ink4b promoter and activate the transcription of the gene in response to IFN- $\beta$  treatment.

In AML patients with inv(16), p15INK4B silencing was found to be caused by inv(16)-encoded core binding factor beta-smooth muscle myosin heavy chain (CBF $\beta$ -SMMHC). CBF $\beta$ -SMMHC was shown to displace RUNX1 from a newly determined CBF site in the promoter of p15INK4B.

## Protein

### Description

p15INK4B transcript encodes two protein isoforms p15 and p15.5 that are functionally indistinguishable. p15.5 is an N-terminally extended variant of p15 initiated from an upstream alternative in frame initiation codon. p15 protein is 138 aa long and its mass is 14.72 KDa.

p10 transcript encodes the shorter variant. The protein consists of 78 aa only and its mass is 10 KDa. It shares a similar NH2 terminus to p15 but contains a different basic COOH terminus that is translated from the p15Ink4b intronic region (Figure 2).

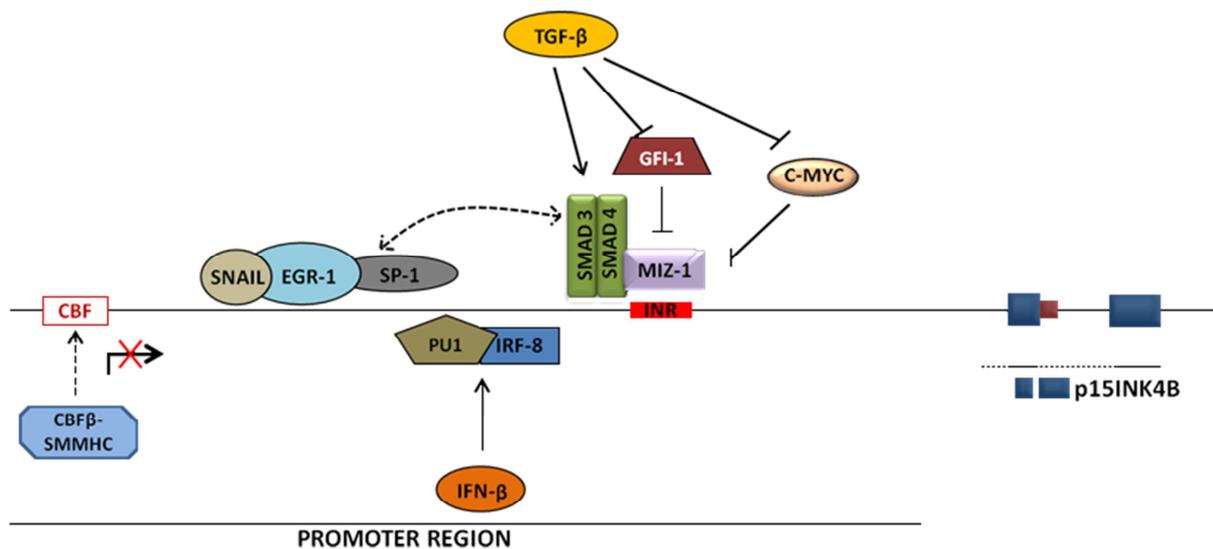


Figure 2.

### Expression

p15INK4B is expressed at very low levels under normal physiological conditions. Its expression seems to be lineage restricted. In bone marrow cells the highest level of p15INK4B is mainly detected in maturing monocytes/macrophages and lymphocytes. The gene expression has also been reported to be normally up-regulated during megakaryocytic differentiation. Increased expression of p15INK4B is also detected during stress and senescence of cells.

### Localisation

Nucleus and cytoplasm.

### Function

I- Function in the cell cycle.

p15INK4B belongs to the INK4 family of protein kinase inhibitors named for their high and exclusive specificity towards the catalytic activity of cyclin dependent kinases 4 (CDK4) or 6 (CDK6). Structural studies have demonstrated that the protein performs its inhibitory activity by allosteric competition with the D-type cyclins to bind CDK4/6 kinases and prevents the formation of active CDK4/6-cyclin-Ds complexes. This keeps the retinoblastoma protein (RB), which is downstream of this pathway, in its hypophosphorylated state. Hypophosphorylated RB binds and inactivates the E2F transcription factors required for the transcriptional activation of genes necessary for entry into the S phase of the cell cycle and DNA synthesis.

Three other members of the INK4 family of CDK inhibitors: p16INK4A, p18INK4C and p19INK4D are encoded by unique genes and share roughly 40% homology. They have similar protein structure characterized by the presence of four ankyrin-like motif tandem repeats that are predicted to be engaged in protein-protein interactions.

II- Function during hematopoietic cell differentiation. Another role for p15INK4B during differentiation of

early hematopoietic progenitors has also been described. In knockout mice, loss of p15INK4B was shown to favor the differentiation of common myeloid progenitors (CMP) into granulocyte macrophage progenitors (GMP) resulting in an imbalance between the myeloid and the erythroid compartments.

III- Function during cellular senescence.

Cellular senescence is accompanied by hallmark features that include the up-regulation of cell cycle inhibitors like p15INK4B, p16INK4A and p21CIP. When overexpressed, p15INK4B engages the RB pathway to promote a stable senescent state which has been shown to occur in part through a process that involves alterations in heterochromatin and the stable silencing of E2F target genes.

Another mechanism that has been described is the inactivation of c-MYC which results in the induction of p15INK4B expression and correlates with the global changes in heterochromatin structure known to be associated with cellular senescence.

### Homology

p15INK4B is highly conserved. Its sequence in homo sapiens is > 85% similar to bos taurus, mus musculus and rattus norvegicus; and > 70% similar to gallus gallus.

### Mutations

#### Note

Intragenic p15INK4B mutations are highly infrequent.

### Implicated in

#### Various hematological disorders and malignancies

#### Note

p15INK4B is frequently epigenetically silenced in leukemias, myelodysplastic syndromes and

myeloproliferative diseases by mechanisms involving aberrant DNA methylation and/or histone modifications. These diseases are subcategorized by the French-American-British (FAB) co-operative group, based on the percentage of blast cells in bone marrow and peripheral blood, degree of cytopenia, and in accordance to the direction of differentiation along the myeloid or lymphoid lineages as well as the degree of maturation of the hematopoietic cells.

### **Myelodysplastic syndromes (MDS)**

#### **Disease**

Myelodysplastic syndromes are heterogeneous clonal hematologic disorders characterized by dysplasia of the myeloid bone marrow cells accompanied with peripheral blood cytopenia and increased risk of transformation to acute myeloid leukemia (AML). MDS transforms into AML once the percentage of blasts in the bone marrow has exceeded 30% (FAB). MDS can arise in patients de novo (primary MDS), or following chemotherapy or exposure to toxins (secondary MDS). According to the Leukemia and Lymphoma Society reports, MDS most commonly affects males aged 70 and above, and is considered to be a disease of the elderly. About 11000 new cases are diagnosed each year, resulting in an incidence rate of 4 cases per 100000 population for both genders.

#### **Prognosis**

p15INK4B is silenced by promoter hypermethylation in > 50% of MDS cases. Levels of p15INK4B methylation increase as the disease progresses and provide a marker that can predict occurrence of AML.

### **Chronic myelomonocytic leukemia (CMML)**

#### **Disease**

The defining features of CMML are an absolute monocytosis in peripheral blood ( $> 1 \times 10^9/L$ ), increased numbers of monocytes in bone marrow, a variable degree of dysplasia and less than 5% and 20% of blasts in peripheral blood and bone marrow, respectively. There are two types of CMML: proliferative and dysplastic. Roughly half of CMML diagnosed patients have an elevated white blood cell count commonly associated with hepatomegaly and splenomegaly (myeloproliferative form of the disease). Patients lacking these features are generally considered to have the myelodysplastic form of the disease.

#### **Prognosis**

Hypermethylation is found in up to 60% of CMML cases and correlates with a more aggressive form of disease. Experimentally, a LysMCre mouse model was developed in which p15INK4B gene is deleted specifically in cells of the myeloid lineage, to better mimic the loss of the gene expression the way it is observed in humans. The mice develop non-reactive monocytosis of the peripheral blood as well as increased myeloid blast progenitors in the bone

marrow. In this way the mice develop symptoms that closely resemble CMML in human patients.

### **Acute myeloid leukemia (AML)**

#### **Disease**

AML is the most common type of leukemia among adults with 14000 new cases diagnosed each year, and with 9000 deaths per year in the United States. AML classification into ten different subtypes was originally defined by the FAB cooperative group according to the direction of differentiation along the different myeloid lineages as well as the degree of maturation of the cells. However, AML exemplifies a genetically heterogeneous cancer with more than a hundred genetic aberrations implicated in the disease.

#### **Prognosis**

Despite the great genetic and phenotypic heterogeneity of AML, hypermethylation of the p15INK4B promoter region (CpG island) is found to occur in up to 80% of AML cases across all FAB subtypes. It correlates with a loss of p15INK4B expression, poor prognosis and shorter survival time in patients. The p15INK4B methylation status in AML patients in clinical remission is now monitored and used as a reliable prognostic marker for relapse. These findings were further experimentally confirmed in a conditional knockout mouse model where myeloid-specific gene inactivation resulted in an increased susceptibility to retrovirus-induced myeloid leukemia.

### **Acute lymphoblastic leukemia (ALL)**

#### **Disease**

There are about 4000 new cases of ALL in the United States each year. It appears most often in children younger than age 10. ALL is the most common leukemia in children. However, it can appear in people of any age. About one-third of cases are adults.

#### **Prognosis**

In B and T acute lymphoblastic leukemia the p15INK4B promoter methylation as well as deletion of the entire locus has been reported.

### **Chronic leukemia**

#### **Disease**

Chronic leukemia can be subdivided into two subtypes, chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL). CLL is primarily an adult disease; it is very rare in children and young adults. The median age of diagnosis is 72 years, and about 60% of patients are male. In the United States, about 15000 people are diagnosed with CLL each year. This disease is also commonly referred to as B-cell chronic lymphocytic leukemia (B-CLL).

#### **Prognosis**

Promoter hypermethylation has been reported in a small subset of B-CLL (11%) at all stages of the disease. In CML, silencing of p15INK4B either by

deletion or hypermethylation of its promoter was not found to be a very frequent event.

### ***Glioblastoma multiforme (GBM)***

#### **Disease**

GBM is the most common and very aggressive brain tumor in adults. It involves glial cells and accounts for more than 50% of parenchymal brain tumors approximately 20% of all intracranial tumors. Glioblastoma growth is characterized by a high motility of tumor cells that display broad chemoresistance leading to frequent post-surgical tumor recurrence. It is one of the most dreaded cancer diagnoses due to its poor prognosis and the limited treatment options, with the median survival duration after diagnosis varying from 6 months to 2 years.

#### **Prognosis**

Homozygous deletion of the p15INK4B/p14ARF/p16INK4A locus on chromosome 9p21.3 is a signature genetic event that drives the pathogenesis of GBM. The deletion of this locus is the most common homozygous deletion present in GBM (> 75% of samples). Specific p15INK4B promoter methylation was also detected in 37% of patients diagnosed with glioblastoma and it correlated with shorter survival.

### ***Hepatocellular carcinoma (HCC)***

#### **Disease**

HCC is a primary malignancy of the liver that mostly arises secondary to hepatitis B or C viral infections. Outcome of the disease is poor, because only 10 - 20% of hepatocellular carcinomas can be removed completely using surgery, and the cancer is usually deadly within 3 to 6 months.

#### **Prognosis**

The suppression of the C-MYC oncogene induces cellular senescence in diverse tumor types including hepatocellular carcinoma and correlates with increased p15INK4b expression. In primary HCC, p15INK4B promoter is hypermethylated in about 50% of the cases, and homozygous deletions of both p16INK4A and p15INK4B have been reported in 30% HCC patients and cell lines. This suggests that p15INK4B might be contributing to human hepatocarcinogenesis through a pathway associated with cellular senescence.

## **References**

Jen J, Harper JW, Bigner SH, Bigner DD, Papadopoulos N, Markowitz S, Willson JK, Kinzler KW, Vogelstein B. Deletion of p16 and p15 genes in brain tumors. *Cancer Res.* 1994 Dec 15;54(24):6353-8

Tsubari M, Tiihonen E, Laiho M. Cloning and characterization of p10, an alternatively spliced form of p15 cyclin-dependent kinase inhibitor. *Cancer Res.* 1997 Jul 15;57(14):2966-73

Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. *Leukemia.* 1998 Jun;12(6):845-59

Aoki E, Uchida T, Ohashi H, Nagai H, Murase T, Ichikawa A, Yamao K, Hotta T, Kinoshita T, Saito H, Murate T. Methylation status of the p15INK4B gene in hematopoietic progenitors and peripheral blood cells in myelodysplastic syndromes. *Leukemia.* 2000 Apr;14(4):586-93

Fuxe J, Raschperger E, Pettersson RF. Translation of p15.5INK4B, an N-terminally extended and fully active form of p15INK4B, is initiated from an upstream GUG codon. *Oncogene.* 2000 Mar 23;19(13):1724-8

Jeffrey PD, Tong L, Pavletich NP. Structural basis of inhibition of CDK-cyclin complexes by INK4 inhibitors. *Genes Dev.* 2000 Dec 15;14(24):3115-25

Latres E, Malumbres M, Sotillo R, Martín J, Ortega S, Martín-Caballero J, Flores JM, Cordon-Cardo C, Barbacid M. Limited overlapping roles of P15(INK4b) and P18(INK4c) cell cycle inhibitors in proliferation and tumorigenesis. *EMBO J.* 2000 Jul 3;19(13):3496-506

Malumbres M, Ortega S, Barbacid M. Genetic analysis of mammalian cyclin-dependent kinases and their inhibitors. *Biol Chem.* 2000 Sep-Oct;381(9-10):827-38

Teofili L, Morosetti R, Martini M, Urbano R, Putzulu R, Rutella S, Pierelli L, Leone G, Larocca LM. Expression of cyclin-dependent kinase inhibitor p15(INK4B) during normal and leukemic myeloid differentiation. *Exp Hematol.* 2000 May;28(5):519-26

Amati B. Integrating Myc and TGF-beta signalling in cell-cycle control. *Nat Cell Biol.* 2001 May;3(5):E112-3

Schmidt M, Koller R, Haviernik P, Bies J, Maciag K, Wolff L. Deregulated c-Myb expression in murine myeloid leukemias prevents the up-regulation of p15(INK4b) normally associated with differentiation. *Oncogene.* 2001 Sep 27;20(43):6205-14

Seoane J, Pouponnot C, Staller P, Schader M, Eilers M, Massagué J. TGFbeta influences Myc, Miz-1 and Smad to control the CDK inhibitor p15INK4b. *Nat Cell Biol.* 2001 Apr;3(4):400-8

Staller P, Peukert K, Kiermaier A, Seoane J, Lukas J, Karsunky H, Möry T, Bartek J, Massagué J, Hänel F, Eilers M. Repression of p15INK4b expression by Myc through association with Miz-1. *Nat Cell Biol.* 2001 Apr;3(4):392-9

Teofili L, Martini M, Di Mario A, Rutella S, Urbano R, Luongo M, Leone G, Larocca LM. Expression of p15(ink4b) gene during megakaryocytic differentiation of normal and myelodysplastic hematopoietic progenitors. *Blood.* 2001 Jul 15;98(2):495-7

Wolff L, Schmidt M, Koller R, Haviernik P, Watson R, Bies J, Maciag K. Three genes with different functions in transformation are regulated by c-Myb in myeloid cells. *Blood Cells Mol Dis.* 2001 Mar-Apr;27(2):483-8

Narita M, Núñez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell.* 2003 Jun 13;113(6):703-16

Tessema M, Länger F, Dingemann J, Ganser A, Kreipe H, Lehmann U. Aberrant methylation and impaired expression of the p15(INK4b) cell cycle regulatory gene in chronic myelomonocytic leukemia (CMML). *Leukemia.* 2003 May;17(5):910-8

Qin Y, Liu JY, Li B, Sun ZL, Sun ZF. Association of low p16INK4a and p15INK4b mRNAs expression with their CpG islands methylation with human hepatocellular carcinogenesis. *World J Gastroenterol.* 2004 May 1;10(9):1276-80

Schmidt M, Bies J, Tamura T, Ozato K, Wolff L. The interferon regulatory factor ICSP/IRF-8 in combination with PU.1 up-

regulates expression of tumor suppressor p15(Ink4b) in murine myeloid cells. *Blood*. 2004 Jun 1;103(11):4142-9

Aggerholm A, Holm MS, Guldberg P, Olesen LH, Hokland P. Promoter hypermethylation of p15INK4B, HIC1, CDH1, and ER is frequent in myelodysplastic syndrome and predicts poor prognosis in early-stage patients. *Eur J Haematol*. 2006 Jan;76(1):23-32

Markus J, Garin MT, Bies J, Galili N, Raza A, Thirman MJ, Le Beau MM, Rowley JD, Liu PP, Wolff L. Methylation-independent silencing of the tumor suppressor INK4b (p15) by CBFbeta-SMMHC in acute myelogenous leukemia with inv(16). *Cancer Res*. 2007 Feb 1;67(3):992-1000

Papageorgiou SG, Lambropoulos S, Pappa V, Economopoulou C, Kotsioti F, Papageorgiou E, Tsirogitis P, Dervenoulas J, Economopoulos T. Hypermethylation of the p15INK4B gene promoter in B-chronic lymphocytic leukemia. *Am J Hematol*. 2007 Sep;82(9):824-5

Wu CH, van Riggelen J, Yetil A, Fan AC, Bachireddy P, Felsher DW. Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. *Proc Natl Acad Sci U S A*. 2007 Aug 7;104(32):13028-33

Peters G. An INKlination for epigenetic control of senescence. *Nat Struct Mol Biol*. 2008 Nov;15(11):1133-4

Solomon DA, Kim JS, Jean W, Waldman T. Conspirators in a capital crime: co-deletion of p18INK4c and p16INK4a/p14ARF/p15INK4b in glioblastoma multiforme. *Cancer Res*. 2008 Nov 1;68(21):8657-60

Yu W, Gius D, Onyango P, Muldoon-Jacobs K, Karp J, Feinberg AP, Cui H. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature*. 2008 Jan 10;451(7175):202-6

Basu S, Liu Q, Qiu Y, Dong F. Gfi-1 represses CDKN2B encoding p15INK4B through interaction with Miz-1. *Proc Natl Acad Sci U S A*. 2009 Feb 3;106(5):1433-8

Bies J, Sramko M, Fares J, Rosu-Myles M, Zhang S, Koller R, Wolff L. Myeloid-specific inactivation of p15Ink4b results in monocytosis and predisposition to myeloid leukemia. *Blood*. 2010 Aug 12;116(6):979-87

Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. *PLoS Genet*. 2010 Dec 2;6(12):e1001233

Hu CT, Chang TY, Cheng CC, Liu CS, Wu JR, Li MC, Wu WS. Snail associates with EGR-1 and SP-1 to upregulate transcriptional activation of p15INK4b. *FEBS J*. 2010 Mar;277(5):1202-18

Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, Xiong Y. Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene*. 2011 Apr 21;30(16):1956-62

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