ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Promoter Hypermethylation In Liver Cancer.

Sanyogita Rai, Gunjan Raghav, Mohini Kaushik, Pooja Rai, Jyotsna Yadav, Anchal Bamal, and Abhimanyu Kumar Jha*.

Department of Biotechnology, Faculty of life sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India.

ABSTRACT

Cancer has become one of the fatal diseases since past few decades. There are many types of cancer and liver cancer is one of them. It is asymptomatic at its initial stage and can be non- curative at severe stage. HCC (Hepatocellular carcinoma) occurs in liver tissue or tissue or cells associated with it such as cholangiocytes. Common symptoms include- nausea, vomiting, decrease in appetite, abdominal swelling, enlarged liver, weight loss, weakness, fatigue etc. Liver cancer can originate due to various factors and epigenetic mechanism is one of them. Epigenetic changes are the alteration in genetic structure of an individual due to some external environmental factors. Histone modification, DNA methylation , RNA associated silencing. Promoter hypermethylation of CpG island can lead to silencing of various tumor suppressor genes that can cause liver cancer. These hypermethylated genes can be utilized as a biomarker for the diagnosis of HCC. Some of these TSGs(Tumor Suppressor Genes)include PCHD8, DENN2D, CDN2KA, ZHX2, RASSF1, DOK1, NAT2, SOX-17, SMPD3, FHL, TIP 30,CDH 1, RASGRF 1, FBP 1 etc.

Keywords: HCC, cholangiocytes, DNA methylation, promoter hypermethylation, biomarkers, TSGs

https://doi.org/10.33887/rjpbcs/2019.10.3.64

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Liver cancer is a type of cancer that originates in the liver(Ferlay et al., 2010; Jemal et al., 2011) and affected by primary liver cancer (hepatocellular carcinoma), or through cancer which spread to the other part of body and then spread to the liver. Several types of cancer can form in the liver and the most common type of liver cancer is Hepatocellular carcinoma (HCC), it is a type of Primary liver cancer which begins in the liver cell (hepatocytes). Other types of liver cancer such as hepatoblastoma, intrahepatic cholangiocarcinoma and the most liver cancer is secondary or metastatic. Cholangiocarcinoma is arising from cholangiocytes and it is the second most common primary liver malignant tumor. About 10% to 20% of liver cancer in the liver is intrahepatic cholangiocarcinoma, they start in the bile ducts outside the liver. There are many symptoms occur in liver cancer but they do not appear in early stages. The symptoms of liver cancer may differ for each person. Common symptoms of cancer that develops in the liver may includeNausea and vomiting not associated with other known conditions, decrease in appetite or a feeling of fullness after a small meal, abdominal swelling, enlarged liver (hepatomegaly) felt as a under the ribs on the right side, weight loss not associated with changes in diet, general weakness or fatigue etc that contributes to HCC metastasis, invasion, DNA methylation, expression of noncoding RNAs and histone modification. All these changes are associated with initiation and progression of HCC. Hypermethylation of promoter CpG islands is an epigenetic mechanism of gene silencing Primary liver cancer (HCC) is an invasive tumor that occurs in the chronic liver disease such as hepatitis B virus (HBV) and hepatitis C virus (HCV) (Perz et al.,2006), hemochromatosis and cirrhosis. The risk factors prompt chronic liver damage usually leading to cirrhosis, which is present in HCC about 80-90% of patients.(EI serag et al.,2011) Liver cancer is the second leading cause of cancer, about 700,000 deaths take place due to liver cancer.(Lovet et al.,2003) Primary liver cancer or hepatocellular carcinoma (HCC) is the seventh most common cancer in females and fifth most common cancer in males (Ferlay et al.,2010; Jemal et al., 2011) . Liver cancer is emerging as one of the fastest spreading cancers in India. India sees about 3-5 cases of liver cancer per 1, 00,000 people which translates to 30,000- 50,000 new cases per year.

Hepatocellular Carcinoma (HCC) is predominant malignancies with high fatality rate. In HCC and other wide range of tumors, specific promoter hypermethylation and global hypomethylation have been associated with inactivation of tumor-suppressor genes (TSGs) and genomic instability. Different epigenetic changes cing that are involved in a wide range of human cancers, including HCC (Herceg et al., 2007)

EPIGENETICS

Epigenetic changes are the alteration in genetic structure of an individual due to some external environmental factors, i.e, there is genetic control by factors other than an individual's DNA sequence, that can influence some diseases and these changes are heritable in humans. They are able to switch genes on or off and determine the transcribed protein. They refer to changes in chromosome that alter gene activity and expression and can be on physiological and phonotypical traits. Three systems are there that can interact with each other to gene silencing (Egger et al., 2004)

Histone Modification

Histone is the core component of chromatin. Histone modification can lead to various altered phenotypical effects. Histone can be modified in two ways either by methylation or acetylation

RNA-associated silencing

When RNA is in the form of antisense transcripts (non-coding RNAs), it can turn off the genes.This phenomenon is also known as RNA interference. (Egger et al., 2004)

DNA Methylation

This is a phenomenon in which DNA is methylated chemically. It is highly specific and occurs in CpG site. CpG site is a region in which a cytosinenucleotide is located next to guanine nucleotide that is linked by phosphate. DNA methyltransferases (DNMTs) are responsible for this phenomenon to occur (Egger et al.,

2004;Robertson.,2002). Methyl group insertion changes structure and appearance of DNA and also modifies gene's interactions within cell's nucleus

PROMOTER HYPERMETHYLATION

Promoter region consists of CpG islands which is methylated and can cause various carcinoma in humans. Liver cancer is one of them .Tumor can be caused by hypermethylation of CpG islands of tumor suppressor genes.

TUMOR SUPPRESSOR GENES THAT ARE PROMOTER HYPERMETHYLATED IN HCC (Hepatocellular carcinoma)

PCDH8

It is a subgroup of cadherin superfamily which plays multiple roles in cell adhesion, proliferation, differentiation and migration. Most of the members of protocadherin family (PCDH10, 17, and 20) are frequently silenced by methylation of promoter in nasopharynx carcinoma, gastric and colorectal cancers or non-small lung cancers. If promoter of cadherin family genes are hypermethylated then it can lead to various types of carcinoma. HCC or liver cancer is one of them. PCDH8 acts as tumor suppressor but on methylation and mutation it is able to changeexpression. Hypermethylation of PCDH8 are more frequently detected in liver cancer but very low in normal liver tissues.Thereby, PCDH8 promoter hypermethylation can serve as an esteemed diagnostic biomarker for liver cancer.It has been shown that methylation of PCDH8 is significantly correlated with the level of AFP.AFP is an alpha fetoprotein which is a protein substance produced by liver cells.It is one of the first alpha-globulins appeared in mammalian sera during embryo development and dominant serum protein in early embryonic stage. Being a serum marker, AFP level is broadly used for diagnosis as well as surveillance of liver cancer. During the early stage of liver cancer patients, AFP level may be normal upto 40%(low sensitivity). Elevation in the level of AFP,i.e., greater than 400 ng/ml will be considered to have diagnostic biomarker for liver cancer.It has been studied that PCDH8 promoter methylation seems to more frequently occur in patients with AFP level greater than 50ng/ ml that aids in early diagnosis of liver cancer by combining AFP level and PCDH8 methylation status. Promoter hypermethylation of PCDH8 is also associated with tumor size,tumor differentiation,OS (overall survival) and PFS (Progression free survival).OS is the length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease are still alive.PFS is the length of time during and after the treatment of a disease, such as cancer, that a patient lives with the disease but it does not get worse.Tumor size, tumor differentiation are elevated due to methylated PCDH8.OS and PFS is shorter in methylated PCDH8 individuals than unmethylated PCDH8 individuals.

DENN2D

The DENN (Differentially Expressed in Normal and Neoplastic cells) domain is a poorly characterized protein module conserved throughout evolution, with DENN domain proteins found in species as diverse as humans. There are 18 genes encoding DENN domain-containing proteins in humans. DENN domain proteins interact with Rab GTPases provided the first insight into the superfamily of monomeric G-proteins.Rab ,like other GTPases , switch between two confirmations as inactive form of it bound to GDP and active form of it bound to GTP. A GDP/GTP exchange factor (GEF) catayses the conversion from inactive form to active form.In the active state, they recruit effectors that control multiple aspects of membranetrafficking.DENN domain acts as a GEF for Rab.The DENN2D family is the only example wherein the DENN domain is found in the C-terminal region.DENND2 protein acts as GEF for Rab9a/b. Rab9 functions in retrograde trafficking of the mannose phosphate receptor from late endosomes to the trans-Golgi network , and consistently, depletion of DENND2D disturb this trafficking process.Gene DENN2D is located on chromosome 1p13.3, encodes a protein, which suppresses the proliferation and tumorigenicity of non-small cell lung cancer cells .Downregulation of DENN2D leads to hepatocarcinoma it can occur due to hypermethylation of its promoter.The expression level of DENN2D m RNA is significantly lower in liver cancer tissues than in corresponding normal tissues.

CDN2KA

Itis a tumor suppressor gene and encodes p16 which is a cyclin-dependent kinase inhibitor and located in chromosome 9.It plays an important role in cell cycle regulation by ceasing the progression of cell from G1 phase to S phase and hence acts as a tumor suppressor which aids in prevention of cancers.This CDN2KA reports to be inactivated through extensive CpG methylation in hepatitis virus induced HCC. Epigenetic changes of CDKN2A evaluating the methylation status of CpG islands on the first exon as this gene generates many transcript variants that differ in their first exons. It has also been indicated that methylation of CpG islands at the 5'end of the first exon of CDN2KA which may serve as a specific biomarker for HCC as it can be frequently found in HCC patients.(Matsuda et al.,1999;Csepregi et al.,2010)

ZHX2

It encodes Zinc fingers and homeoboxes protein 2 and located in chromosome 8q24.13 .They are nuclear homodimeric and ubiquitous transcriptional repressors. It has been also revealed that ZHX family is involved in the expression of number of nuclear factor –Y(NF-Y) or)regulated genes via an organized transcription network.It has been found that hypermethylation mediates silencing of ZHX2 gene which may take part in development of HCC.The expressionlevel of ZHX2 mRNA in HCC is low in HCC compared with adjacent noncancerous tissue samples, but there may be no significant difference in the expression between low- and high-grade HCCs, indicating that the negative expression of ZHX2 mRNA is related to the carcinogenesis of HCC and might have no relationship to progression. Methylated ZHC2 lost the transcriptional repressor activity which results inuncontrolled proliferation of liver cells by the loss of regulation of NF-Y mediated genes. Perhaps the ZHX2 gene may be regarded as a tumor suppressor(Kawata H.,2003)

RASSF1 (Ras Association Domain-containing protein 1)

Ras association domain-containing protein 1 is a protein in humans that encoded by the RASSF1 gene, located at 3p21.31, appoximately 11,000 bp, and contains eight exons. RASSF1A tumor suppressor gene is ubiquitous in human liver cancer, often silenced through epigenetic mechanism in a variety of human malignancies and it is one of the most inactivated proteins identified in human cancer. The epigenetic inactivation of genes often involves the methylation of CpG islands in their promoters.RASSF1A contains a RAS associationdomain. RASSF1A regulates many biological processes that include apoptosis, microtubule stability and cell cycle progression. (Palakurthy et al., 2009).RASSF1A, has properties compatible with a tumor suppressor function. Moreover, the gene appears to suffer frequent transcriptional inactivation in tumor cells due to aberrant promoter methylation (Burbee et al., 2001;Dammann et al., 2000)progenitors cause HCC in humans. As gene silencing cannot lead to perform its normal function and hence cancer can occur frequently.

SOX-17

SRY-box containing gene 17 (SOX-17) has role in the WNT/β-catenin pathway and acts as a tumor suppressor gene that stops the transcription of activated β-catenin. WNT oncogene plays a crucial role in liver carcinogenesis and has been shown to correlate with poor survival .Promoter hypermethylation of SOX-17 results in silencing of SOX-17 gene which causes overexpression of WNT. Silencing of SOX-17 gene through promoter hypermethylation results in WNT overexpression.(Chelis et al.,2013)

DOK1(Docking protein 1)

DOK protein is enzymatically scaffolding proteins which provide a docking platform for the assembly of multimolecular signaling complexes. Docking protein 1 is encoded by the DOK1 gene in human. The protein encoded by this gene is a part of a signal transduction pathway downstream of receptor tyrosine kinases. Docking protein 1 is localised on human chromosome 2p13.1, which is frequently rearranged in many human tumors. Docking protein 1 is constitutively tyrosine phosphorylated in hematopoietic which isolated from the chronic myelogenous leukemia (CML) patients. DOK1 contains a putative plecksrin homology domain at the amino terminus(Nelms et al,1998). Hypermethylation of DOK1 was found in the majority (88%) of the HCC

cases. The level of DOK1 methylaion in HCC samples was higher in the group of younger patients.(Nelms et al.,1998;Ling Y et al.,2005)

NAT2

NAT2 gene encodes an enzyme N-acetyltransferase 2 (arylamine N-acetyltransferase) .This enzymes activates and deactivates arylamine and hydrazine drugs and carcinogens.NAT2 gene is located on chromosome 8p22. Hypermethylation of this gene can ssive behavior in hepatocellular carcinoma (HCC).

SMPD3

SMPD3 is located in chromosome 16q22. It is a tumor suppressor gene which can be inactivated or silenced by promoter hypermethylation in HCC patients.SMPD3 encodes an enzyme sphingomyelinase which breaks sphingomyelin to produce ceramide via the phosphoinositide-3-kinase/AKT pathway and ceramide has roe in growth arrest, cell differentiation and apoptosis. Loss of SMPD3 impairs apoptosis and increase phosphorylation of AKT which exhibits down regulated expression in numerous primary HCCs

FHL 1

Four and a half LIM domains protein 1 is a protein that in humans is encoded by the FHL1 gene and located on human chromosome Xq26. It involves in regulation of cell proliferation, differentiation and apoptosis and also functions in skeletal and cardiac muscle growth.(Morgan et al.,1999).It is a tumor suppressor gene on chromosome X and has a high risk to be affected as a single hit of genetic and/or epigenetic changes on only one active allele can lead to complete inactivation of FHL1 gene. Its tumor suppressor effect can occur via multiple mechanisms that includes the activation of the transforming growth factor- β- l like andthe mitogen activated protein kinase signaling pathways and protein interaction with zonula occludens-1 , hypoxia- inducible factor 1- α and estrogen receptor- α(Niu et al.,2012;Sakashita et al.,2008;Ding et al., 2009;Shen et al.,2006)

TIP 30

It encodes Tat interacting protein 30. It is a tumor suppressor gene or metastasis suppressor gene. It plays an important role in predisposing of hepatoblastoma cells to programmed cell death through regulating the expression levels of these genes. Promoter hypermethylation of TIP30 cause abnormal expression of tip30 protein in Hepatocellular carcinoma. CpG island methylation of Tip30 gene was ion not found in normal liver tissues, whereas it was detected in 47%of the tumor tissues and 20% of the adjacent tissues. These depict that hypermethylation of Tip30 gene might be a frequent event in HCCs.(Chen X et al.,2010)

CDH1

It encodes E-cadherin which helps in cell adhesion, transmitting chemical signals, control cell maturation and movement and regulates various genes .It acts as a tumor suppressor genes. Promoter hypermethylation of CDH1 gene can lead to downregulation of CDH1 and due to which early development of HCC can occur and allelic deletion of it contribute to malignant progression of HCC.The level of E-cadherin expression may contribute to the determination of the characteristics of HCCs.(Ghee Y et al.,2005)

FBP1

It is a protein in humans which is encoded by the FBP1 gene. Fructose-1,6-bisphosphatase catalyzes the hydrolysis of fructose-1,6 bisphosphate to fructose-6-phosphate and inorganic phosphate. This reaction is an important regulatory site of gluconeogenesis. The human FBP1 locus resides on chromosome 9q22.3; FBP1 gene contains 7 exons and spans more than 31 kb. The expression and DNA methylation of FBP1 in primary HCC was low expressed in 80% human hepatocellular carcinoma, 66.7% liver cancer cell lines, which was well correlated with its promoter methylation status. Fructose-1, 6- diphosphatase deficiency is associated with hypoglycemia and metabolic acidosis. FBP1 underwent promoter CpG hypermethylation-associated silencing in human liver, that FBP1 functions as a TSG to suppress cancer cell growth through the induction of G2-M

phase cell cycle arrest and an increase in ROS generation levels. Fructose-1, 6-diphosphatase deficiency is associated with hypoglycemia and metabolic acidosis.(Chen M et al.,2011)

CONCLUSION

It has been reported that the TSGs could be utilized for identifying epigenetic markers in future, for the better treatment of cancer patients across over the world. They could be prove to a potent source in early detection of tumor that will be enhancing or increasing the rate of recovery. Consequently, biomarkers that can also be termed as tumor markers have a great biological and clinical importance in future to fight cancer.

REFERENCES

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM Int J Cancer. 2010 Dec 15; 127(12):2893-917.

[2] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D CA Cancer J Clin. 2011 Mar-Apr; 61(2):69-90.

[3] Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006; 45:529–38

[4] El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011; 365:1118–27

[5] Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003; 362:1907–17.

[6] L. Ma, M.-S. Chua, O. Andrisani, and S. So, "Epigenetics in hepatocellular carcinoma: an update and future therapy perspectives," World Journal of Gastroenterology, vol. 20, no. 2, pp. 333–345, 2014

[7] Herceg Z. Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. Mutagenesis. 2007; 22:91–103.

[8] Tischoff I, Tannapfe A. DNA methylation in hepatocellular carcinoma. World J Gastroenterol. 2008; 14:1741–8

[9] .Egger, G., *et al*. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429, 457–463

[10] Hu X, Sui X, Li L, Huang X, Rong R, Su X, Shi Q, Mo L, Shu X, Kuang Y, Tao Q,He C. Protocadherin 17 acts as a tumour suppressor inducing tumour cellapoptosis and autophagy, and is frequently methylated in gastric andcolorectal cancers. J Pathol. 2013; 229(1):62-73.

[11] Imoto I, Izumi H, Yokoi S, Hosoda H, Shibata T, Hosoda F, Ohki M, HirohashiS, Inazawa J. Frequent silencing of the candidate tumor suppressor PCDH20by epigenetic mechanism in non-small-cell lung cancers. Cancer Res. 2006;66(9):4617-26.

[12] Zhang X, Li HM, Liu Z, Zhou G, Zhang Q, Zhang T, Zhang J, Zhang C. Loss of heterozygosity and methylation of multiple tumor suppressor genes onchromosome 3 in hepatocellular carcinoma. J Gastroenterol. 2013; 48(1):132-43.

[13] Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. Aberrantmethylation of multiple tumor suppressor genes in aging liver, chronichepatitis, and hepatocellular carcinoma. Hepatology. 2008; 47(3):908-18.

[14] Nishida N, Goel A. Genetic and epigenetic signatures in humanhepatocellular carcinoma: a systematic review. Curr Genomics. 2011;12(2):130-7.

[15] Summers T, Langan RC, Nissan A, Brucher BL, Bilchik AJ, Protic M, DaumerM, Avital I, Stojadinovic A. Serum-based DNA methylation biomarkers incolorectal cancer: potential for screening and early detection. J Cancer. 2013;4(3):210-6.

[16] Cheng Zhang, Yunfei Peng, Fan Yang, Ruixi Qin, Wenjun Liu, Cuijuan Zhang.PCDH8 is frequently inactivated by promoter hypermethylation in liver cancer : Diagnostic and clinical significance. Journal of cancer 2016; 7(4):446-52

[17] Bloethner S, Mould A, Stark M, Hayward NK. Identification of ARHGEF17,DENND2D, FGFR3, and RB1 mutations in melanoma by inhibition of nonsense-mediated mRNA decay. Genes Chromosomes Cancer. 2008;47:1076–85.

[18] Marat AL, Dokainish H, McPherson PS. DENN domain proteins: regulators of Rab GTPases. J Biol Chem. 2011;286:13791–800.

[19] Ling B, Zheng H, Fu G, Yuan J, Shi T, Chen S, et al. Suppression of non-small cell lung cancer proliferation and tumorigenicity by DENND2D. Lung Cancer. 2013;79:104–10.

[20] Baek MJ, Piao Z, Kim N-G, Park C, Shin E-C, Park J-H, Jung H-J, Kim CG, KimH: p16 is a major inactivation target in hepatocellular carcinoma.*Cancer* 2000, 89:60-68.

[21] Matsuda Y, Ichida T, Matsuzawa J, Sugimura K, Asakura H: p16INK4 isinactivated by extensive CpG methylation in human hepatocellularcarcinoma. *Gastroenterology* 1999, 116:394-400.

[22] Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC: 5' CpG islandmethylation is associated with transcriptional silencing of the tumorsuppressor p16/CDKN2/MTS1 in human cancers. *Nat Med* 1995,1:686-692.

[23] Schneider-Stock R, Boltze C, Peters B, Hopfner T, Meyer F, Lippert H,Roessner A: Differences in loss of p16INK4 protein expression bypromoter methylation between left- and right-sided primarycolorectal carcinomas. *Int J Oncol* 2003, 23:1009-1013.

[24] Kawata H, Yamada K, Shou Z, et al. Zinc-fingers and homeoboxes(ZHX) 2, a novel member of the ZHX family, function as atranscriptional repressor. *Biochem J.* 2003;373:747-757.

[25] Lee S, Lee HJ, Kim JH, et al. Aberrant CpG islandhypermethylation along multistep hepatocarcinogenesis. *Am JPathol.* 2003;163:1371-1378.

[26] Chae HD, Yun J, Bang YJ, et al. Cdk2-dependentphosphorylation of the NF-Y transcription factor is essentialfor the expression of the cell cycle–regulatory genes and cellcycle G1/S. *Oncogene.* 2004;23:4084-4088.

[27] . Donninger H, Vos MD, Clark GJ. The RASSF1A tumor suppressor. J Cell Sci. 2007; 120:3163–72.

[28] . Saulnier A, Vaissiere T, Yue J, Siouda M, Malfroy M, Accardi R, et al. Inactivation of the putative suppressor gene DOK1 by promoter hypermethylation in primary human cancers. Int J Cancer. 2012; 130:2484–94.

[29] . Hesson, L. B., Cooper, W. N. and Latif, F. The role of RASSF1A methylation in cancer. Dis. Markers 2007*,* 73-*87.*

[30] Palakurthy, R. K., Wajapeyee, N., Santra, M. K., Gazin, C., Lin, L., Gobeil, S., Green, M. R. Epigenetic silencing of the RASSF1A tumor suppressor gene through HOXB3-mediated induction of DNMT3B expression. Molec. Cell 2009; 36: 219-230,

[31] Burbee, D. G., Forgacs, E., Zöchbauer-Müller, S., Shivakumar, L., Fong, K., Gao, B., Randle, D., Kondo, M., Virmani, A., Bader, S. et al. *(*2001). Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J. Natl. Cancer Inst.* **93***,* 691-699

[32] . Dammann, R., Li, C., Yoon, J.-H., Chin, P. L., Bates, S. and Pfeifer, G. P. Epigenetic inactivation of a RAS association domain family protein from lung tumour suppressor locus 3p21.3. Nat. Genet 2000. 25, 315-319.

[33] Carpino N, Wisniewski D, Strife A, Marshak D, Kobayashi R, Stillman B, Clarkson B "p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells". Cell 1997; 88 (2): 197–204

[34] Nelms K, Snow AJ, Noben-Trauth K "Dok1 encoding p62(dok) maps to mouse chromosome 6 and human chromosome 2 in a region of translocation in chronic lymphocytic leukemia". Genomics 1998; 53 (2): 243–5.

[35] Ling Y, Maile LA, Badley-Clarke J, Clemmons DR ."DOK1 mediates SHP-2 binding to the alphaVbeta3 integrin and thereby regulates insulin-like growth factor I signaling in cultured vascular smooth muscle cells". J. Biol. Chem 2005; 280 (5): 3151–8.

[36] Chelis L, Balgouranidou I, Koukaki T; Assessment of SOX-17 gene promoter hypermethylation in hepatocellular carcinoma.Journal of clinical oncology2013, 31, no.15

[37] Chen X,Cao X,DDong W,Luo S,Suo Z,Jin Y;Expression of TIP30 Iis downregulated in human colorectal carcinoma 2010;55(8):2219-26.

[38] Ng EK, Lee SM, Li HY, Ngai SM, Tsui SK, Waye MM, Lee CY, Fung KP. Characterization of tissue-specific LIM domain protein (FHL1C) which is an alternatively spliced isoform of a human LIM-only protein (FHL1) J Cell Biochem. 2001;82:1–10.

[39] Morgan MJ, Madgwick AJ. The LIM proteins FHL1 and FHL3 are expressed differently in skeletal muscle. Biochem Biophys Res Commun. 1999;255:245–250

[40] Niu C, Liang C, Guo J, Cheng L, Zhang H, Qin X, Zhang Q, Ding L, Yuan B, Xu X, et al. Downregulation and growth inhibitory role of FHL1 in lung cancer. Int J Cancer. 2012;130:2549–2556.

[41] Sakashita K, Mimori K, Tanaka F, Kamohara Y, Inoue H, Sawada T, Hirakawa K, Mori M. Clinical significance of loss of Fhl1 expression in human gastric cancer. Ann Surg Oncol. 2008;15:2293–2300

[42] Ding L, Wang Z, Yan J, Yang X, Liu A, Qiu W, Zhu J, Han J, Zhang H, Lin J, et al. Human four-and-a-half LIM family members suppress tumor cell growth through a TGF-beta-like signaling pathway. J Clin Invest. 2009;119:349–361

[43] Shen Y, Jia Z, Nagele RG, Ichikawa H, Goldberg GS. SRC uses Cas to suppress Fhl1 in order to promote nonanchored growth and migration of tumor cells. Cancer Res. 2006;66:1543–1552

[44] Ghee Young Kwon, Byung Chul Yoo, Kwang Cheol Koh, Jae Won Cho, Won Sang Park, Cheon Keul Park.Promoter hypermethylation of E-cadherin in hepatocellular carcinomas and dysplastic nodules. Journal of Korean medical science 2005; 20(2):242-247

[45] Mingquan chen, Jianbin zhang, Ning li, Zhiping Qian, Mengqi zhu, Qian Li, Jianming zheng, Xinyu wang.Promoter hypermethylation mediated downregualation of FBP1 in human hepatocellular carcinoma and colon cancer. Plos one 6(10): e25564

.