

Novelty of *Axin 2* and lack of *Axin 1* gene mutation in colorectal cancer: a study in Kashmiri population

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Abstract Colorectal cancer is (CRC) one of the leading causes of mortality and morbidity. Various genetic factors have been reported to be involved in the development of colorectal cancers including *Axin* gene. *Axin*, a major scaffold protein, plays an important role in various bio signaling pathways. We aim to study mutational pattern of *Axin* gene in colorectal cancer patients of Kashmiri population. The paired tumor and adjacent normal tissue specimens of 50 consecutive patients with CRC were used in our study. The DNA preparations were evaluated for the occurrence of *Axin 1* and *Axin 2* gene mutations by direct DNA sequencing. We analyzed exon 1a, 1b, 1c, 2, 4, 6, and 10 of *Axin 1* and exon 7 of *Axin 2*. In this study, we found a novel mutation of G>T (GCT>TCT) transversion in exon 7 of *Axin 2* gene at codon G695T (p.alanine > serine) at a frequency of 6% (3/50). In the same exon of *Axin 2* gene a

single nucleotide polymorphism (SNP) was detected in codon L688L (CCT>CTT) at a frequency of 36% (18/50). In exon 1c of *Axin 1* a SNP was detected at codon D726D (GAT>GAC) at a frequency of 62.5% (31/50). Both the SNPs were synonymous hence do not lead to change of amino acid. Although *Axin 1* and *Axin 2* gene mutations have been found to be involved in the development of colorectal cancers, it seems to be a relatively rare event in Kashmiri population. However, an interesting finding of this study is the novelty of *Axin 2* gene mutations which may be a predisposing factor in ethnic Kashmiri population to CRC.

Keywords Mutation · *Axin* · Colorectal carcinoma · Kashmiri population

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Introduction

Colorectal cancer (CRC) is a common malignancy and the most common cause of mortality and morbidity among men and women in western countries. In 2010, 142,570 new cases were diagnosed, and 51,370 deaths occurred due to colorectal cancer [1]. In Kashmir, colorectal cancer is showing an increasing trend and ranks at 4th position among various common cancers prevalent here in Kashmir valley (Arshad et al. unpublished data). CRC is implicated by peculiar customary dietary habits prevalent in Kashmir since centuries [2]. Colorectal cancer is a multifactorial disease involving various environmental and dietary factors [3]. Apart from external factors, genetic factors also play role in the development of CRC.

CRC develops as a result of accumulated genetic events involving mutational activation of proto-oncogenes and loss of function mutations which inactivate tumor-suppressor

genes [4]. Axis inhibition protein (Axin 1) and its homologue Axin 2 (also known as conductin) are tumor suppressor genes and acts as master scaffold protein. Axin has pleiotrophic effect on various signaling pathways. One of its key functions is to negatively regulate the activity of the Wnt pathway. The Wnt/Wingless biological signaling pathway plays an important role in both embryonic development and tumorigenesis [5].

Apart from Wnt signaling pathway, Axin plays a role in TGF- β signaling and SAPK/JNK (stress activated protein kinase/Jun N Terminal kinase) [6]. TGF- β signaling regulates several cellular functions, including proliferation, differentiation, migration, and apoptosis [7]. TGF- β also plays an important role in carcinogenesis. Axin has been shown to regulate the TGF- β signaling pathway by acting as an adaptor for Smad3, one of the TGF- β effectors. Upon receptor activation, Smad3 bound to Axin is efficiently phosphorylated by TGF- β receptor 1 and dissociates from the Axin complex. Thus, Axin may facilitate the phosphorylation and transcriptional activity of Smad3 [8]. TGF- β is one of the anti-oncogenic factors that inhibit nuclear β -catenin signaling in the Wnt pathway. Although Axin is a negative regulator of the Wnt signaling pathway, it facilitates TGF- β signaling. These results indicate that Axin has a dual function in signal transduction—acting as a negative regulator of the Wnt signaling pathway and as a positive regulator of the TGF- β signaling pathway [9].

The SAPK/JNK signal transduction pathway is activated in cells in response to stress. It is also involved in many normal physiological processes including tissue morphogenesis, cell proliferation, cell survival, and cell death [10]. Over expression of Axin 1 in cells stimulates SAPK/JNK and can induce apoptosis through the activation of MEKK (mitogen activated protein/extracellular regulated kinase kinase) [11, 12]. The effect of Axin on the induction of apoptosis is mediated by SAPK/JNK activation and the destabilisation of β -catenin [13].

Alterations in both *Axin 1* and *Axin 2* have been detected in several different tumors. Mutations are found in most *Axin* domains including the APC (RGS-Regulator of G protein Signaling) and β -catenin-binding domains. *Axin* sequence variants have also been found in colon, ovarian, endometrioid, adenocarcinoma, and HCC cell lines. Biochemical and functional studies have shown that these mutations interfere with the binding of GSK3 and that they also alter the interaction between Axin and two upstream activators of TCF-dependent transcription, Frat1, and DVL [14]. *AXIN 1* mutations have been reported in a variety of human carcinomas including endometrioid adenocarcinomas, Hepatocellular carcinomas, and colorectal cancers [15–18], suggesting that *Axin 1* may be involved in the development of these tumors. *Axin 2* has been shown to develop frame shift mutations in mononucleotide repeat

sequences located in exon 7, in colorectal cancer, with defective DNA mismatch repair (MMR) [19].

Keeping in view of the heavy burden of CRC patients and lack of any such study in this region, we aim to investigate the mutational profile of *Axin 1* and *Axin 2* gene in colorectal cancer patients of ethnic Kashmiri population.

Materials and methods

Study design

This prospective study was conducted at the Sher-I-Kashmir Institute of Medical Sciences (SKIMS) in Kashmir, India between 2009 and 2011. The Medical Ethical Committee of SKIMS Deemed University approved the study. All patients signed the written informed consent.

Participants

A total of 50 patients of CRC were included in this study. Tumor and adjacent normal tissue samples were resected in the General surgery department (SKIMS). Median age at the time of diagnosis was 52 years (range 30–75); and male: female ratio was 1:1. Clinico pathological characteristics of patients are given in Table 1. In this study 29 (58%) patients had cancer in the rectum while colon cancer accounted for 21 (42%) CRC cases. 33 (61%) cases of CRC were well differentiated and 17 (34%) were poorly/moderately differentiated. 31 (62%) of CRC patients belonged to rural area and 19 (38%) to urban area. Almost all the patients had attended the hospital with a clinical presentation of bleeding per rectum. The resected tissue specimens were brought fresh from the theater to the Department of Histopathology. The diagnostic slides were reviewed by a panel of 2 expert pathologists to confirm the diagnosis and only the confirmed cases of CRC were included in our study.

Deoxyribonucleic acid (DNA) extraction

Samples of both the tumor and adjacent normal tissue were collected. Samples were snap-frozen immediately and stored at -80°C . DNA from both types of tissue was extracted by salting out procedure using nuclease lysis buffer (NaCl, Na_2EDTA , SDS) chloroform, *protenaise K*, and salted out by ammonium acetate. Extracted DNA was dissolved in tris-EDTA buffer for further use.

Polymerase chain reaction (PCR)

Exons 1, 2, 4, 6, and 10 of the *Axin 1* gene and exon 7 of *Axin 2* gene were amplified using previously described specific

Table 1 Primers used for amplification of different exons of *AXIN 1* and *AXIN 2* genes

Exon No.	Primer sequence	AT°C	AP(bp)
Exon 1a	F 5'-AGGGTGCAGCGCTCATTGTT-3'	58	311
	R 5'-AATGCAGTGACTCAGCCCACT-3'		
Exon 1b	F 5'-GGATCTGGACCTGGGGTATG-3'	58	387
	R 5'-ATAGTGGCCTGGATTTTCGGT-3'		
Exon 1c	F 5'-ATCATGAAGCAGCTGATCG-3'	60	405
	R 5'-GAGGTGAGTACAGAAAGTGGAC-3'		
Exon 2	F 5'-CTGTTGGCAGGCTGTCACT-3'	60	224
	R 5'-GTCCGTGAGGGACTGGGTA-3'		
Exon 4	F 5'-TTTAGCCTGTGACCTTTCAAC-3'	58	253
	R 5'-ATCCCGGCGGCAAGAA-3'		
Exon 6	F 5'-CACCGGAGGCCAGGGCGACT-3'	68	256
	R 5'-TGGCAAAGCAGGCCCCACGA-3'		
Exon 10	F 5'-CACGCCGTCCCCTGCCAC-3'	63	260
	R 5'-GACACCCGTGCCCGCAA-3'		
Exon 7	F 5'-AACCCAGTTTCTTTCTTCT-3'	54	320
	R 5'-ATCCCTGCCTCAACCTA-3'		

AT Annealing temperature, AP Amplified product size, F forward, R reverse

primers [17, 20]. The primers details are given in Table 1. PCR amplification was carried out in a 50 μ L volume container with 50 ng of genomic DNA, 1 \times PCR buffer containing 1.5 mM MgCl₂, 100 μ M each of dATP, dGTP, dTTP, dCTP, and 1.5 U of Taq DNA polymerase (Biotools; Madrid, Spain), and 1 μ M of forward and reverse primers (Genescript; Piscataway, NJ, USA). The thermal conditions were set as: initial denaturation at 95°C for 5 min, 35 cycles of 94°C for 40 s, a specific annealing temperature (Table 1) for 40 s and 72°C for 40 s, and final extension at 72°C for 7 min. The PCR products were run on 2% agarose gel and analyzed under an ultraviolet illuminator.

PCR products were purified manually by eluting amplicons from agarose gel with the help of a chaotropic agent, Sodium Iodide (NaI). Eluted amplicons bind to DNA binding resin (glass milk), while as agarose and other contaminants are washed away. The DNA was then eluted in Tris–EDTA buffer. The purified PCR amplicons were used for direct DNA sequencing, using the automated DNA sequencer ABI prism 310.

Data analysis

Chi-square was used to compare the *Axin 1* and *Axin 2* gene SNPs with the clinico-epidemiological characteristics. *P*-value < 0.05 was considered statistically significant.

Results

In this study, DNA sequencing was used to analyze the exons 1a, 1b, 1c, 2, 4, 6, and 10 of *Axin 1* and exon 7 of *Axin 2* in a series of 50 CRC patients. Clinico-pathological

parameters of all patients are given in Table 2. No previously reported mutations were detected in any of the analyzed exons of *Axin 1* and *Axin 2* genes in CRC patients except two SNPs mentioned below. However, an interesting finding of this study was that we detected a novel mutation of G>T (GCT>TCT) transversions in exon 7 of *Axin 2* gene at codon G695T (p.alanine >serine) which has not been reported till date. This G695T novel mutation was further confirmed by reverse sequence of the same samples (Fig. 1a). This mutation was found at a frequency of 6% (3/50). Among these three patients, two were chronic smokers with mean age of 57 years. They had well differentiated adenocarcinoma. Clinico-pathological characteristics of patients having novel mutation are given in Table 3.

Table 2 Clinico-epidemiological characteristics of the CRC patients

Clinico-epidemiological parameters	Subgroup	Cases (n = 50)
Grade(differentiation)	WD	33 (66%)
	MD/PD	17 (34%)
Location	Colon	29 (58%)
	Rectum	21 (42%)
Dwelling	Rural	31(62%)
	Urban	19 (38%)
Age	<50	19 (38%)
	\geq 50	31 (62%)
Sex	Male	24 (48%)
	Female	26 (52%)
Smoking status	Never	21 (42%)
	Ever	29 (58%)

WD well differentiated, MD moderately differentiated, PD poorly differentiated

Fig. 1 **a** Partial nucleotide sequences in Exon 1c of the normal (*left*) and mutants in exon 1c of the *Axin 1* gene codon (GAT → GAC). *Red arrow* points toward base change in mutants with respect to normal sequence. **b** Partial nucleotide sequences in Exon 7 of the normal (*left*) and mutants in exon 7 of the *Axin 2* gene codon (CCT → CTT). *Red arrow* points toward base change in mutants with respect to normal sequence. **c** Partial nucleotide sequences in Exon 7 of normal (*left*) and of the mutants in (*right*) of the *Axin 2* gene codon (GCT>TCT) Partial reverse sequence of the same mutation (*below*). *Red arrow* points toward base change in mutants with respect to normal sequence

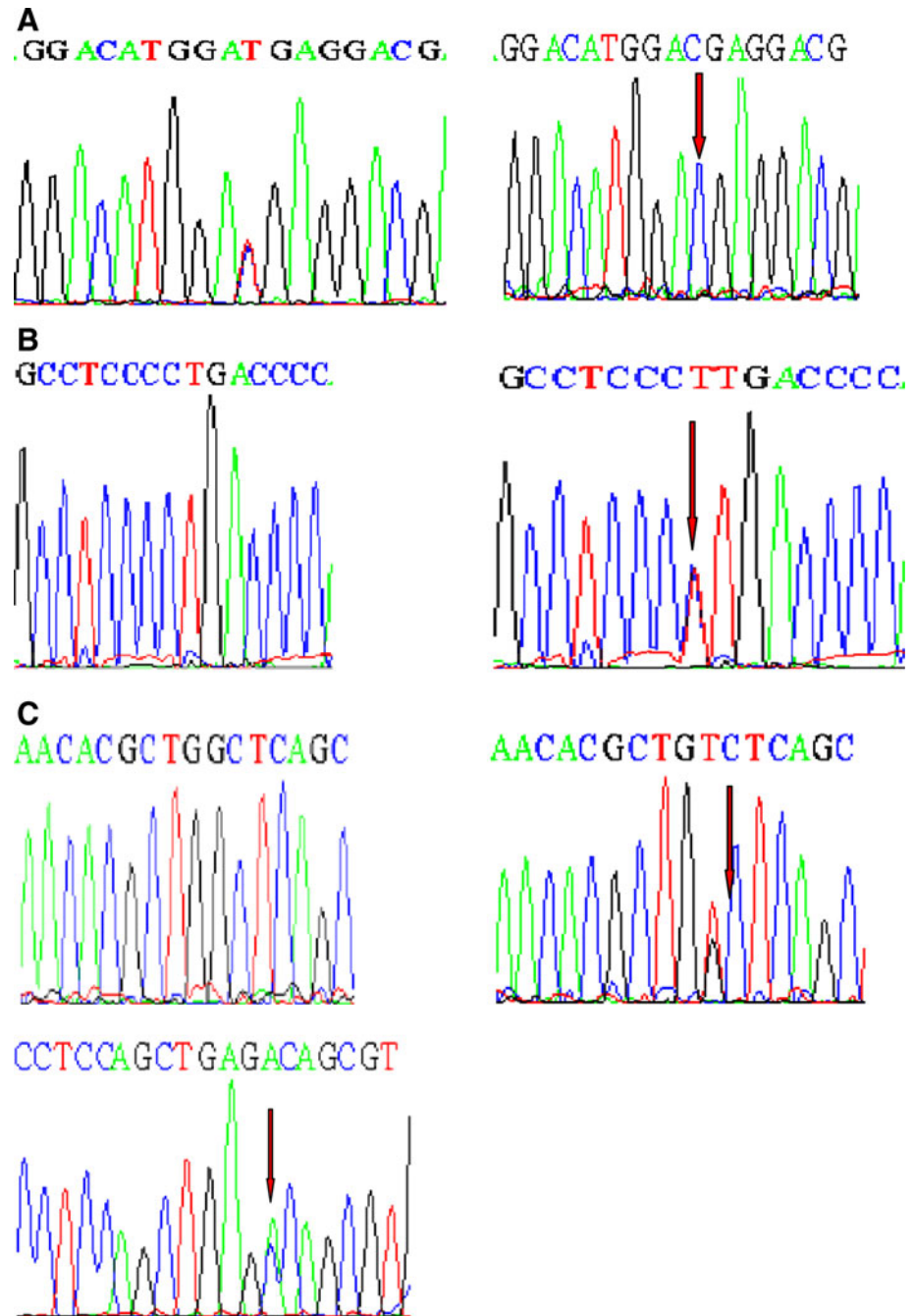


Table 3 Clinico-epidemiological characteristics of the patients with novel mutation in *Axin 2* gene

Sample code	Age	Gender	S.status	Dwelling	Location	HPG	C.change	A.A change	N.change
CRC 29	50	Male	c.smoker	Urban	A. Colon	WD	A695S GCT → TCT	Alanine → serine	2397G → T
CRC 32	65	Male	c.smoker	Urban	Rectum	WD	A695S GCT → TCT	Alanine → serine	2397G → T
CRC 38	57	Female	c.smoker	Rural	colon	WD	A695S GCT → TCT	Alanine → serine	2397G → T

S.status Smoking status, *C.smoker* chronic smoker, *HPG* Histopathological grade, *WD* well differentiated, *A.A change* Amino acid change, *N.change* nucleotide change, *C.change* codon change, *A. Colon* ascending colon

Table 4 Single nucleotide changes in *Axin 1* and *Axin 2* genes in CRC patients

Gene/ Exon	N.change	C.change	A.A change	Frequency
Axin 1	1134	D726D	Asp → Asp	31/
Exon 1c	T → C	GAT → GAC		50(62.5%)
Axin 2	2376	L688L	Leu → Leu	18/50(36%)
Exon 7	C → T	CCT → CTT		

A.A change amino acid change, C.change codon change, N.change nucleotide change. (Transcript ID of axin 1 gene ENSG00000103126, NCBI Reference Sequence NC_000016.9) (Transcript ID of axin 2 gene ENSG00000168646 NCBI Reference Sequence: NC_000017.10)

In the same exon of *Axin 2* gene a single nucleotide polymorphism (SNP) of C>T transition was detected in codon L688L (CCT>CTT) at a frequency of 18/50(36%) (Fig. 1b). In exon 1c of *Axin 1*, we detected a SNP of T>C transition at codon D726D (GAT>GAC) at a frequency of 31/50 (62.5%) (Fig. 1c). These SNPs were synonymous and do not lead to any change of amino acid. Table 4 shows the changes in nucleotides of *Axin 1* and *Axin 2* genes observed in our study. No significant association of

these SNPs was found with any Clinico epidemiological characteristics (Table 5).

Our team had previously analyzed same series of CRC samples for loss of heterozygosity (LOH) in Deleted in colorectal cancer gene (DCC) using two microsatellite markers (M2 and VNTR). Loss of heterozygosity was observed in 26 out of 80 (32.5%) at VNTR and 19 (23.75%) at D18S8-M2 region. The combined frequency of LOH of DCC gene of two markers aggregated to 56.25% (45 of 80) of CRC cases (Data under revision).

Discussion

Axin, a major Scaffold protein, serves as vital mediator for cellular signaling. It plays an architectural role in integrating incoming signals to downstream effectors which in turn manifest biological functions. Axin, named for its inhibitory role of axis specification in vertebrates, is a multidomain protein and regulates a variety of signaling pathways and biological functions [21]. As Axin has the ability to down regulate β -catenin protein levels, it can be regarded as a tumor suppressor [22]. Axin possesses multiple functional domains. It has an RGS (Regulator of G

Table 5 Clinico-epidemiological Characteristics of the CRC Patients with single nucleotide polymorphism

Variables	Cases (n = 50)	SNP at codon 726GAT>GAC AXIN 1 gene		OR (95%CI)	P-value	SNP at codon 688CCT>CTT AXIN 2 gene		OR (95%CI)	P-value
		Variant allele 31 (62.5%)	Wild allele 19 (38%)			Variant allele 18(36%)	Wild allele 32 (64%)		
Grade									
WD	33 (66%)	20 (60%)	13 (39.4%)	0.8 (0.3–3.4)	0.77	14 (42%)	19 (58%)	2.4 (0.27–3.7)	0.19
MD/PD	17 (34%)	11 (64%)	06 (35.3%)			04 (24%)	13 (76%)		
Age Group									
<50	19 (38%)	11(57.9%)	08 (47.4%)	0.8 (0.3–3.2)	0.63	06 (32%)	13 (68%)	0.7 (0.3–1.2)	0.10
≥50	31 (62%)	20 (64.5%)	11 (35.5%)			12 (39%)	19 (61%)		
Gender									
Male	24 (48%)	14 (58%)	10 (42%)	0.7 (0.3–3.1)	0.63	08 (33.3%)	16 (67%)	0.8 (0.3–1.17)	0.7
Female	26 (52%)	17 (65%)	09 (35%)			10 (38.4%)	16 (62%)		
Smoking									
Never	21 (42%)	12 (57%)	09 (43%)	0.7 (0.3–3.2)	0.62	08 (38%)	13 (62%)	1 (0.3–1.2)	0.79
Ever	29 (58%)	19 (66%)	10 (34%)			10 (34%)	19 (66%)		
Residence									
Rural	31 (62%)	19 (61%)	12 (39%)	1.7 (0.3–3.1)	0.33	11 (35%)	20 (65%)	0.9 (0.3–3.28)	0.9
Urban	19 (38%)	09 (47%)	10 (53%)			07 (37%)	12 (63%)		
Tumor site									
Colon	29 (58%)	19 (66%)	10 (34%)	1.4 (0.3–3.1)	0.54	11 (38%)	18 (62%)	1.2 (0.3–3.25)	0.7
Rectum	21 (42%)	12 (57%)	09 (43%)			07 (33%)	14 (67%)		

χ^2 was used to calculate P-value. P-value ≤ 0.05 was considered significant

protein Signaling) domain that is conserved in the protein family of regulators of G protein signaling, near its NH2 terminus. At the C terminus, Axin contains another domain named DIX (Disheveled and axin binding domain) that is shared by Dishevelled and a recently identified Wnt regulator called Ccd1. In addition, Axin contains numerous domains for interaction with multiple other proteins than APC and Dvl/Ccd1 that bind to the RGS and to the DIX domain, respectively [21]. Owing to its capacity to bind multiple proteins, it plays a role in more than one signaling pathway. Most of the *Axin 1* mutations in colorectal cancer occur between exon 1 and 5, where the APC, GSK3, and β -catenin binding domains are located [6]. Mutations in *Axin 2* have been found in approximately 20% of mismatch repair deficient colorectal tumors [19].

In our study, we report single nucleotide change of T>C in exon 1c of *Axin 1* at codon 726 and of C>T at codon L688L in exon 7 of *Axin 2* genes in colorectal cancer patient. These sequence variations are silent and do not lead to change in amino acid. In exon 7 of *Axin 2*, we observed a single nucleotide change which was found to be a novel mutation of G>T at codon 695. This mutation was found at a frequency of 6% in CRC patients in our population, a finding that has been never reported previously leading to change of amino acid from alanine to serine. Sarawera et al. 2006 reported 11% (5/47) C>T transversion at codon L688L in exon 7 of *Axin 2* gene in CRC Samples and 8% (2/26) cell lines. Our study is not in accordance with the previous studies [18, 23]. SNPs of *Axin 1* and *Axin 2* gene reported in this study were compared with those recorded on the National Center for Biotechnology Information (NCBI) Single Nucleotide Polymorphism database (dbSNP) (<http://www.ncbi.nlm.nih.gov/SNP/>).

LOH of DCC gene is associated with microsatellite instability characterized by the expansion or contraction of short repeated DNA sequences (microsatellite markers) caused by insertion or deletion of repeated units which depicts possible defect in DNA mismatch repair Genes (MMR) [24]. The samples in which we found novel mutation were informative and showed LOH of DCC gene. This suggests that these samples may have defect in DNA mismatch repair genes. Colorectal cancers with defective MMR genes have been reported to harbor *Axin 2* mutations [19].

Amino acid 695 lies in the domain shared by many proteins like MEKK4, CKI, PIAS, PP2A, and Smad3, thus playing important role in not only Wnt signaling but also C-Jun/SAPK and TGF- β pathway [21]. MEKK4 is an *Axin*-binding protein that mediates *Axin*-induced JNK activation independently of MEKK1 [25]. MEKK4 binds to a region of aa 678–712 which is inside the PP2A binding domain. *Axin* also regulates the effects of Smad3 in the transforming growth factor beta (TGF- β) signaling pathway and functions as an adaptor for Smad3, facilitating its activation by TGF- β

receptors for efficient TGF- β signaling [26]. In the absence of activated TGF- β receptors, *Axin* physically interacted with Smad3 through its C-terminal region located between the β -catenin-binding site and DIX domain lying in the region of aa 508–713, and facilitates the activation of Smad3 by TGF- β receptors [21]. The phosphorylated Smad3 then translocate into the nucleus, where it regulates the transcription of target genes. Although TGF- β can often appear to have different effects in different cell types, its primary effect on colonic epithelial cells is to reduce proliferation and induce differentiation [27]. CKI protein interacts with Amino acid lying in region 508–712 of *Axin*, after binding with *Axin* it enhances β -catenin degradation by acting as priming kinase for GSK-3 β and thus plays crucial role in inhibiting Wnt signaling pathway. PP2A binding domain lies in the region of aa 508–712 of *Axin* gene, it interacts with *Axin* and enhances β -catenin degradation [21].

It seems that change of amino acid from alanine to serine encoded by codon 695 in the region capable of binding to various proteins discussed above could cause a conformational change that somehow renders *Axin* incapable of binding proteins involved in different pathways hence leading to the derangement of Wnt, TGF- β , Jun/SAPK pathways. Aberration in specific binding of these signaling molecules to *Axin* due to the mutation G695T found in our study perhaps may aid in the deregulation of these molecules which have been previously confirmed to be implicated in the development of many cancers [28, 29].

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References

1. American Cancer Society. Cancer Facts & Figures 2010. American Cancer Society, Atlanta
2. Kumar R, Mende P, Wacker CD, Spiegelhalter B, Preussmann R, Siddiqi M (1992) Caffeine-derived *N*-nitroso compounds—I: nitrosatable precursors from caffeine and their potential relevance in the etiology of oesophageal and gastric cancers in Kashmir, India. *Carcinogenesis* 13:2179–2182
3. Gregory LB, Russell GR (2006) Colorectal Cancer Risk Factors. Colorectal Cancer. Armenian Health Network, Health.am. <http://www.health.am/cr/colorectal-cancer/>. Retrieved 16 Jan 2008
4. Vogelstein B, Fearon ER, Hamilton SR, Kem SE, Preisinger AC, Lepper M, Nakamura Y, White R, Smits AMM, Bos IL (1988) Genetic alterations during colorectal tumor development. *N Engl Med* 319:525–532
5. Hong-Tao X, Qiang W, Yang L, Lian-He Y, Shun-Dong D, Yang H, Juan-Han Y, Nan L, En-Hua W (2007) Overexpression of axin downregulates TCF-4 and inhibits the development of lung cancer. *Ann Surg Oncol* 14:3251–3259

6. Salahshor S, Woodgett JR (2005) The links between axin and carcinogenesis. *J Clin Pathol* 58:225–236
7. Massague J (1998) TGF-beta signal transduction. *Annu Rev Biochem* 67:753–791
8. Furuhashi M, Yagi K, Yamamoto H et al (2001) Axin facilitates Smad3 activation in the transforming growth factor beta signaling pathway. *Mol Cell Biol* 21:5132–5141
9. Nishita M, Hashimoto MK, Ogata S et al (2000) Interaction between Wnt and TGF-beta signalling pathways during formation of Spemann's organizer. *Nature* 403:781–785
10. Ip YT, Davis RJ (1998) Signal transduction by the c-Jun N-terminal kinase (JNK) from inflammation to development. *Curr Opin Cell Biol* 10:205–219
11. Zhang Y, Neo SY, Wang X et al (1999) Axin forms a complex with MEKK1 and activates c-Jun NH(2)-terminal kinase/stress-activated protein kinase through domains distinct from Wnt signaling. *J Biol Chem* 274:35247–35254
12. Zhang Y, Neo SY, Han J et al (2000) Dimerization choices control the ability of axin and dishevelled to activate c-Jun N-terminal kinase/stress-activated protein kinase. *J Biol Chem* 275:25008–25014
13. Neo SY, Zhang Y, Yaw LP et al (2000) Axin-induced apoptosis depends on the extent of its JNK activation and its ability to down-regulate beta-catenin levels. *Biochem Biophys Res Commun* 272:144–150
14. Webster MT, Rozycka M, Sara E et al (2000) Sequence variants of the *axin* gene in breast, colon, and other cancers: an analysis of mutations that interfere with GSK3 binding. *Genes Chromosomes Cancer* 28:443–453
15. Wu R, Zhai Y, Fearon ER, Cho KR (2001) Diverse mechanisms of betacatenin deregulation in ovarian endometrioid adenocarcinomas. *Cancer Res* 61:8247–8255
16. Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, Kawasoe T, Ishiguro H, Fujita M, Tokino T, Sasaki Y, Imaoka S et al (2000) *AXIN1* mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of *AXIN1*. *Nat Genet* 24:245–250
17. Taniguchi K, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, Nagorney DM, Burgart LJ, Roche PC, Smith DI, Ross JA, Liu W (2002) Mutational spectrum of β -catenin, *AXIN1*, and *AXIN2* in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 21:4863–4871
18. Li-Hua J, Qiu-Jie S, Wen L, Zhi-Yun YE, Qing L, Sheng-Cai L (2003) Detection of point mutations of the *Axin1* gene in colorectal cancers. *Int J Cancer* 107:696–699
19. Matthias K, Cristian C, Frank T, Helga G, Sonja M, von Arndt H, Karl G, Göran P, Maja P, Svante P (2000) Mutation of *Axin 2* causes colorectal cancer with defective mismatch repair by activating b-catenin/tcf signaling. *Nat Genet* 26(2):146–147
20. Lin T, Guro EL, Tone L, Chieu BD, Gunn IM, Torleiv OR, Ragnhild AL (2005) Genetic and epigenetic changes of components affecting the WNT pathway in colorectal carcinomas stratified by microsatellite instability. *Neoplasia* 7(2):99–108
21. Wen L, Sheng-Caim L (2004) Axin: a master scaffold for multiple signaling pathways. *Neurosignals* 13:99–113
22. Peifer M, Polakis P (2000) Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. *Science* 287:1606–1609
23. Webster MT, Rozycka M, Sara E (2000) Sequence variants of the *axin* gene in breast, colon, and other cancers: an analysis of mutations that interfere with GSK3 binding. *Genes Chromosomes Cancer* 28:443–453
24. Söreide K, Janssen EA, Soiland H, Korner H, Baak JP (2006) Microsatellite instability in colorectal cancer. *Br J Surg* 93:395–406
25. Luo W, Ng WW, Jin LH, Ye Z, Han J, Lin SC (2003) *Axin* utilizes distinct regions for competitive MEKK1 and MEKK4 binding and JNK activation. *J Biol Chem* 278:37451–37458
26. Furuhashi M, Yagi K, Yamamoto H, Furukawa Y, Shimada S, Nakamura Y, Kikuchi A, Miyazono K, Kato M (2001) *Axin* facilitates Smad3 activation in the transforming growth factor beta signaling pathway. *Mol Cell Biol* 21:5132–5141
27. Heldin CH, Miyazono K, ten Dijke P (1997) TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 390:465–471
28. de Caestecker MP, Piek E, Roberts AB (2000) Role of transforming growth factor-beta signaling in cancer. *J Natl Cancer Inst* 92(17):1388–1402
29. LeppaE S, Dirk B (1999) Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* 18:6158–6162