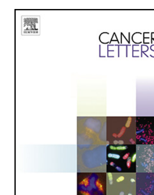
View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Cancer Letters 355 (2014) 1–8

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet



Mini-review

AXIN1 and AXIN2 variants in gastrointestinal cancers

Serina M. Mazzoni [a], Eric R. Fearon [a,b,c,*]

[a] Department of Human Genetics, University of Michigan Medical School, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA
[b] Department of Internal Medicine, University of Michigan Medical School, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA
[c] Department of Pathology, University of Michigan Medical School, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 8 September 2014
Accepted 10 September 2014

Keywords:
Wnt signaling
Beta-catenin
Colon cancer
Hepatocellular carcinoma
Gastric cancer

ABSTRACT

Mutations in the *APC* (*adenomatous polyposis coli*) gene, which encodes a multi-functional protein with a well-defined role in the canonical Wnt pathway, underlie familial adenomatous polypsosis, a rare, inherited form of colorectal cancer (CRC) and contribute to the majority of sporadic CRCs. However, not all sporadic and familial CRCs can be explained by mutations in *APC* or other genes with well-established roles in CRC. The AXIN1 and AXIN2 proteins function in the canonical Wnt pathway, and AXIN1/2 alterations have been proposed as key defects in some cancers. Here, we review *AXIN1* and *AXIN2* sequence alterations reported in gastrointestinal cancers, with the goal of vetting the evidence that some of the variants may have key functional roles in cancer development.
© 2014 Published by Elsevier Ireland Ltd.

Introduction

Somatic mutations in genes functioning in the canonical, or β-catenin-dependent, Wnt pathway are found in approximately 90% of colorectal cancers (CRCs) [1]. These mutations contribute to CRC development by stabilizing the "free" signaling pool of β-catenin via disruption of the "β-catenin destruction complex", which includes the APC (adenomatous polyposis coli) [2–4], AXIN1 [5–7], AXIN2 [8,9], and GSK3β proteins [10,11]. The most common destruction complex defects in CRCs are loss of function mutations in the *APC* tumor suppressor gene, although a subset of CRCs have *CTNNB1* mutations encoding a mutant β-catenin protein that is likely resistant to regulation by the destruction complex [12,13]. While *APC* is the most frequently mutated Wnt pathway tumor suppressor gene, both germline and somatic mutations in the *AXIN1* or *AXIN2* genes have been identified in a subset of CRCs and in several other cancer types. Since the first reports of *AXIN1/2* mutations in cancer, other studies have been completed and *AXIN1/2* sequence polymorphisms in the general population have been better defined. The purpose of this review is to offer a comprehensive update of the reported constitutional and somatic *AXIN1* and *AXIN2* sequence variants described thus far. Previous publications addressing *AXIN1/2* mutations have reviewed selected variants and have sometimes used different *AXIN1/2* reference sequences, making it difficult to compare mutations in a comprehensive fashion. Additionally, since the time of some prior publications, germline sequence variation in control populations has been described in more detail [14]. In-depth evaluation of previously reported and recently reported *AXIN1/2* mutations with reference to consistently annotated AXIN1/2 amino acid sequences and the current list of known polymorphisms will lead to a better understanding of which *AXIN1/2* sequence variants may confer functional consequences for cancer development. While this review specifically addresses *AXIN1/2* mutations in gastrointestinal (GI) cancers, a comprehensive table of *AXIN1/2* sequence alterations reported in non-GI cancers is shown in Appendix: Supplementary Table S1.

The Wnt/β-catenin pathway

The canonical Wnt pathway regulates cell fate during development and cellular homeostasis in adult tissues, and Wnt pathway dysregulation is seen in many cancer types (reviewed in Anastas and Moon) [15]. The canonical Wnt pathway transmits extracellular Wnt signals to the nucleus via effects on β-catenin levels and localization [16]. In the absence of an activating Wnt ligand, a protein complex assembles to phosphorylate β-catenin at multiple residues in its amino-terminal domain [12,13,17,18]. The phosphorylated β-catenin is then recognized by a ubiquitin ligation protein complex and subsequently targeted for degradation by the proteasome [19]. The APC, AXIN1, and AXIN2 proteins are thought to function in the assembly of a β-catenin destruction complex. When an activating Wnt ligand is present, the destruction complex is inhibited and the free, signaling pool of β-catenin can translocate to the nucleus, where β-catenin binds to TCF/LEF transcription factors to modulate the expression of target genes [16,20–22].

* Corresponding author. Tel.: +1 734 764 1549; fax: 1+734-647-7950.
E-mail address: fearon@umich.edu (E.R. Fearon).

http://dx.doi.org/10.1016/j.canlet.2014.09.018
0304-3835/© 2014 Published by Elsevier Ireland Ltd.

AXIN1/2 protein function

There are two AXIN proteins: AXIN1 and AXIN2. In the case of AXIN2, the mouse protein is also known as conductin and the rat AXIN2 protein is also known as Axil. *Axin1* was initially identified as the locus responsible for a series of dominant "kinky" tail mouse phenotypes, all resulting from spontaneous transposon insertions into an exon of *Axin1* [23]. When co-injected with Wnt pathway components into the ventral side of *Xenopus* embryos, *Axin1* mRNA inhibits ectopic axis formation, identifying the protein as a negative regulator of Wnt signaling [24]. A primary function of AXIN1 in the Wnt pathway is in the assembly of the β-catenin destruction complex, thus inhibiting the expression of Wnt- and β-catenin-dependent target genes. Aberrant activation of the Wnt pathway is widely presumed to be a main driver of CRC. Hence, in part because AXIN1 is a negative regulator of β-catenin levels and localization, AXIN1 has been classically thought of as a tumor suppressor protein.

AXIN2 was initially identified based on its yeast-two-hybrid interactions with β-catenin and GSK3β and named for its homology to AXIN1 [8,9]. AXIN2, like AXIN1, acts as a scaffold to help assemble the β-catenin destruction complex, and the two proteins show high similarity to one another in several domains: the Tankyrase binding domain which regulates AXIN protein stability via PARsylation [25]; the RGS domain, which mediates binding to APC [8]; the β-catenin-binding domain [8,9]; and the DIX domain, a dimerization domain named for two proteins which share this motif – Disheveled and AXIN1 [8,26–29]. Similar to AXIN1, AXIN2 negatively regulates β-catenin-dependent Wnt signaling. Injection of *Axil* cDNA in *Xenopus* inhibits the axis duplication caused by activated Wnt signaling, and dorsal cDNA injection results in ventralized embryos [9]. These two AXIN proteins are considered functionally equivalent, as an *Axin2* cDNA rescues the *Axin1*-null lethality in the mouse [30]. However, while the *Axin1* knockout mouse is lethal in embryogenesis at e9.5 [31,32], mice carrying homozygous null mutations in *Axin2* are viable and fertile, with only a mild skull abnormality [33], providing proof that the two genes are not redundant *in vivo*. This difference in phenotype is likely due to the differential expression of the two genes [33]. The expression of *AXIN1* is ubiquitous in various tissues [24], while *AXIN2* shows a more restricted developmental and cell-type-specific expression pattern [9,24]. Uniquely, *AXIN2*, but not *AXIN1*, is a transcriptional target of β-catenin-dependent Wnt signaling [34–36], and *AXIN2* expression is elevated in cancers with activating Wnt pathway mutations. Because of AXIN2's positive regulation by upstream Wnt- and β-catenin-dependent signals and because the AXIN proteins are the least abundant members of the destruction complex, changes in AXIN2 protein levels could be a key negative feedback mechanism for the regulation of Wnt/β-catenin signaling in cells [37].

Colorectal cancer – germline mutations in *AXIN1/2*

Inactivating mutations in the *APC* gene are the most frequent mechanism for activating the Wnt pathway in CRCs, with about 80% of CRCs harboring somatic *APC* mutations [1]. About 0.5–1.0% of CRCs arise in individuals with familial adenomatous polyposis (FAP), who carry an inactivating germline *APC* mutation [38]. Colon and rectal adenomas and CRCs arise in those with FAP, in part, due to somatic genetic or epigenetic inactivation of the remaining wild type *APC* allele, leading to outgrowth of cells with no functional *APC* alleles and dysregulated β-catenin levels. However, germline *APC* mutations only account for a subset of the CRC cases with a hereditary component. *AXIN1* was analyzed as a candidate gene that might harbor CRC-associated germline mutations in a set of 124 UK CRC patients [39]. Several silent mutations were identified as well as seven heterozygous, missense mutations in various exons. Four of these mutations were found only in patients, while the other three were also found in controls, but at lower frequencies. One of the patient-specific mutations has also been described in CRC cell lines [40] and is suggested to interfere with the interaction of AXIN1 and GSK3β [41]. The four patient-specific mutations have since been added to dbSNP, indicating that the variants are found in the general population.

There are only three reported germline mutations in *AXIN2* that have been associated with CRC risk (see Table 1 for more detailed information). The first is a premature stop codon in exon 7 that was found in a family with oligodontia (the absence of multiple adult teeth) and a strong history of CRC [54]. The CRC phenotype in this family was variable, and the age at CRC diagnosis for carriers ranged from 27 to 64 years. A second report of a family with oligodontia and a CRC risk found another premature stop codon, also in exon 7 (see Fig. 1) [55]. This family also had a variable cancer phenotype including one case of early-onset breast cancer. A third germline

Table 1
Summary of *AXIN2* sequence alterations reported in GI cancers.

	Sequence alteration identified*		Cancer type	Reference	Notes
Insertions/deletions	52bp del	delE745-S762	HB cell line D272	[42]	
	del1624	Exons 1–10	HB cell line HUH6	[42]	
	c.2010del12bp	delT672-R675	CRC	[43]	MSS CRC
	c.1925delA	L688X	CRC	[44]	MSI
	c.1926insA	E706X†	CRC	[45]	MSI
	c.1993delG	L688X	CRC	[43]	MSI
	c.1994delG	L688X	CRC, GC	[45] [46], [44], [47],	MSI CRC and GC
	c.1995insG	E706X†	CRC	[45] [46], [44],	MSI
	c.2007delC	L688X	CRC	[48]	MSS
	c.2011delC	L688X	CRC	[45]	MSI
	c.2023delC	L688X	CRC	[45] [44],	MSI
Missense		N412S‡	CRC	[49] [50],	Also seen in controls
		R463C	CRC	[51]	Germline, LOH wt allele
		A603P†,‡	CRC	[49]	Germline, also seen in controls
		R659W‡	HCC	[52]	
		A695S‡	CRC	[53]	
		S738F†,‡	CRC	[49]	Also seen in controls
		S762N‡	CRC, HB cell line D165	[42] [49],	Also seen in controls
Non-sense		R656X	CRC syndrome with oligodontia	[54]	Germline
		W663X	CRC syndrome with oligodontia	[55]	Germline

* Nucleotide and amino acid numbering modified to match modern sequences: NM_004655.3, NP_004646.3.
† These two mutations were found in the same individual, References [49] and [45] respectively.
‡ These variants are now listed in dbSNP (http://www.ncbi.nlm.nih.gov/SNP).

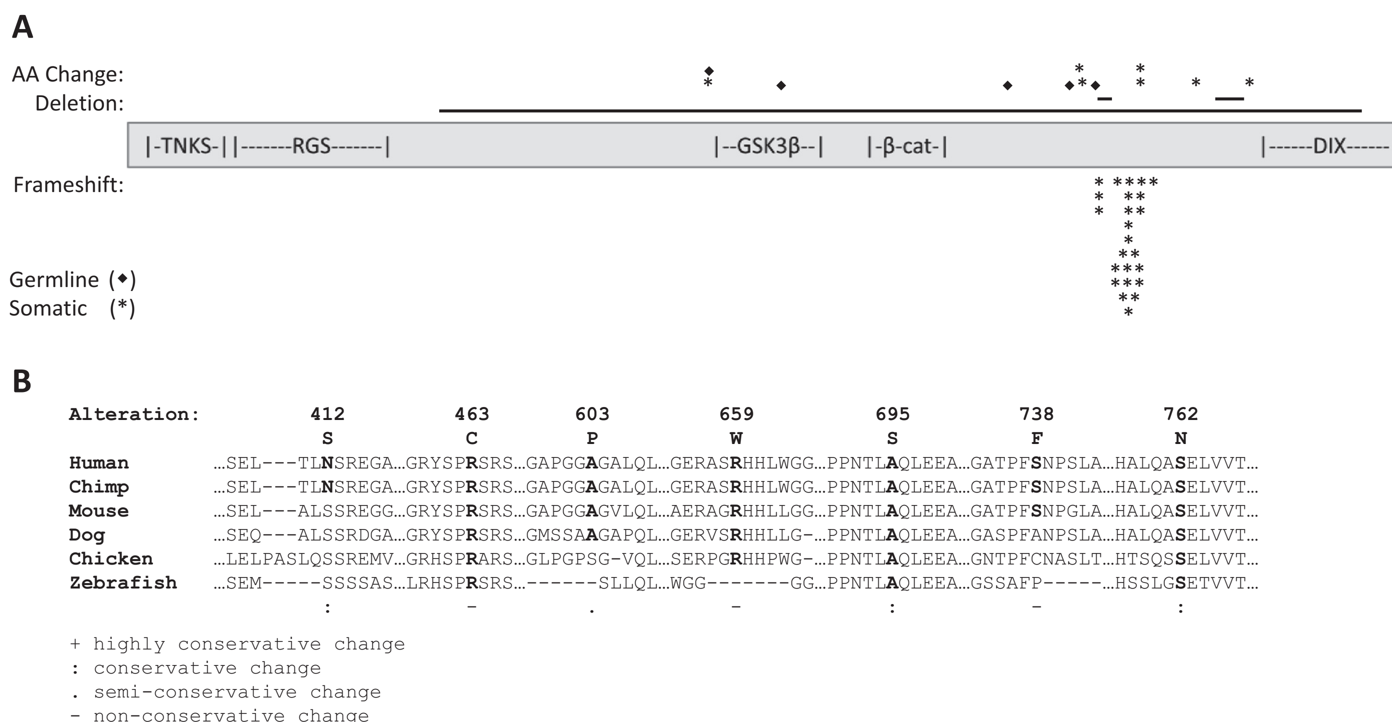


Fig. 1. Location and amino acid conservation of *AXIN2* sequence alterations reported in association with GI cancers. (A) Diagram of the location of germline and somatic *AXIN2* variants implicated in GI cancers. The locations of important functional domains in AXIN2 are indicated. Missense and nonsense variants and deletions are shown above the diagram, frameshifts are shown below. Note the density of frameshifts in the exon 7 mononucleotide repeats region. (B) Amino acid conservation of the missense variants. Conservation designated by Gonnet [56] PAM 250 scores: > 1.5 (+), 0.5 to 1.5 (:), 0.5 to –0.5 (.), <–0.5 (–).

AXIN2 mutation was found in a family with an apparently milder form of familial polyposis, known as attenuated FAP [51]. Of note, this family had no oligodontia or ectodermal dysplasia phenotype. The *AXIN2* mutation in this third family was a missense mutation in exon 5 with possible deleterious effects. However, the role for this *AXIN2* mutation in tumorigenesis seems quite uncertain, as the mutant allele was lost in adenomas and polyps from the proband and his sister. The three reported germline *AXIN2* mutations are heterozygous, suggesting that *AXIN2* mutation could perhaps predispose to cancer via a classical tumor suppressor model, where the second allele is inactivated during tumor formation. However, loss of the wild-type *AXIN2* allele was not seen in the missense mutation family (third family) [51], and the state of the wild type *AXIN2* alleles has not been studied in cancer tissues from individuals carrying the two different *AXIN2* nonsense mutations [54,55]. It is also possible that the nonsense alleles could produce truncated proteins with dominant or haplo-insufficient effects.

A 2005 paper aimed to counter prior reports suggesting that constitutional *AXIN2* sequence variants play an important role in CRC development. Specifically, the paper reported that analysis of 82 probands from families with hereditary CRC revealed 20 *AXIN2* sequence variants in 19 individuals [49]. Silent and intronic mutations were ruled non-pathogenic and none of the identified missense mutations were enriched in patient populations when compared to controls. The authors thus concluded that germline *AXIN2* mutations are not associated with an increased CRC risk. While the findings in this 2005 paper do not support a role for germline *AXIN2* mutations in CRC risk, the collection of familial CRC cases studied was moderate in size and none of the probands had oligodontia. Another paper reporting on analysis of 31 patients with multiple adenomas or CRC found two notable *AXIN2* germline variants in a single patient, but neither variant was clearly pathogenic based on Polyphen analysis: T510T and N412S [50]. Both the T510T and N412S *AXIN2* alleles are now included in dbSNP.

Colorectal cancer – somatic mutations in *AXIN1/2*

Shortly after the identification of a role for AXIN1 in the β-catenin destruction complex, screening of *AXIN1* for mutations in selected patient samples and cell lines from various cancers was undertaken [40]. Two missense alterations were seen in the CRC cell lines studied. The first was S215L, found in the LS513 cell line, which also exhibited loss of heterozygosity (LOH) of the wild type *AXIN1* allele. The second variant, L396M, was found in three cell lines: HCT-8, HCT-15, DLD-1, and the authors chose to follow up on this alteration because of the conservation of the affected amino acid and its location in the GSK3β binding domain (see Fig. 2). Expression of a fragment of the mouse *Axin1* gene with an analogous mutation indeed showed reduced binding to GSK3β, suggesting this variant allele may have functional consequences on the ability of the AXIN1 protein to regulate β-catenin. Because these variants were identified in cell lines, with no matched normal DNA, it was unclear if the *AXIN1* variants are somatic mutations or germline polymorphisms. Both The S215L and L396M variants have since been listed in dbSNP, likely suggesting that these variants represent germline sequence variants, and not somatically acquired mutations.

A 2002 report comparing CRCs harboring mismatch repair pathway (MMR) defects (so-called microsatellite instability-high or MSI-H CRCs) versus CRCs with intact MMR (microsatellite-stable or MSS CRCs) suggested that *AXIN1* mutations may be important in MSI-H CRCs [57]. Out of 33 MSI-H CRC samples studied, eight somatic, missense mutations in *AXIN1* were found in seven tumors. No somatic mutations predicted to alter AXIN1 protein encoding sequences were found in the MSS samples. In the MSI-H CRCs with

AXIN1 somatic mutations, no LOH analysis was done. But, one tumor did show two different *AXIN1* mutations, potentially representing selection for *AXIN1* bi-allelic defects, perhaps akin to the situation seen for some tumor suppressor genes. Another study of *AXIN1* mutations in 54 CRC samples reported five missense mutations and one nonsense mutation [58]. No matching normal tissue or blood samples were available to determine if these were somatic or germline changes. *AXIN2* was not assessed in either study [57,58].

A study by Liu et al. reported that 11 of 45 MSI-H CRCs tested had somatic mutations in *AXIN2* [45]. All of the mutations were frameshifts in exon 7 mononucleotide tracts that were predicted to lead to premature stop codons in the *AXIN2* open reading frame. In 60 CRCs with intact MMR, none contained an exon 7 *AXIN2* frameshift. Only two of the eleven MMR-defective CRCs with *AXIN2* frameshifts showed loss of the wild-type allele, not unexpectedly because LOH is infrequent throughout the genome of MMR-defective CRCs [59]. Assuming the remaining *AXIN2* allele was not silenced by epigenetic mechanisms in the remaining 9 of the 11 MMR CRCs with a frameshift mutation in one allele, the absence of demonstrable bi-allelic *AXIN2* defects suggests that if the *AXIN2* variants were key in promoting colorectal tumorigenesis, they might have done so via dosage-dependent effects and/or through some potential gain-of-function of the truncated AXIN2 proteins encoded. The authors did not rule out nonsense-mediated decay for AXIN2 transcripts with premature stop codons. Of some interest, they did show that overexpression of a truncated AXIN2 protein, predicted to result from the frameshifts, promoted β-catenin accumulation in cells in culture, consistent with a possible gain-of-function role for the truncated AXIN2 protein.

In an analysis of 39 sporadic MSI-H CRCs, frameshifts at *c.1994* or *c.1995* in the exon 7 polyG-tract of *AXIN2* were found in eight tumors [46]. The connection between *AXIN2* exon 7 frameshifts and MMR-deficient CRCs was further supported by a study of 310 CRCs [44]. Sequencing of exon 7 revealed heterozygous frameshift

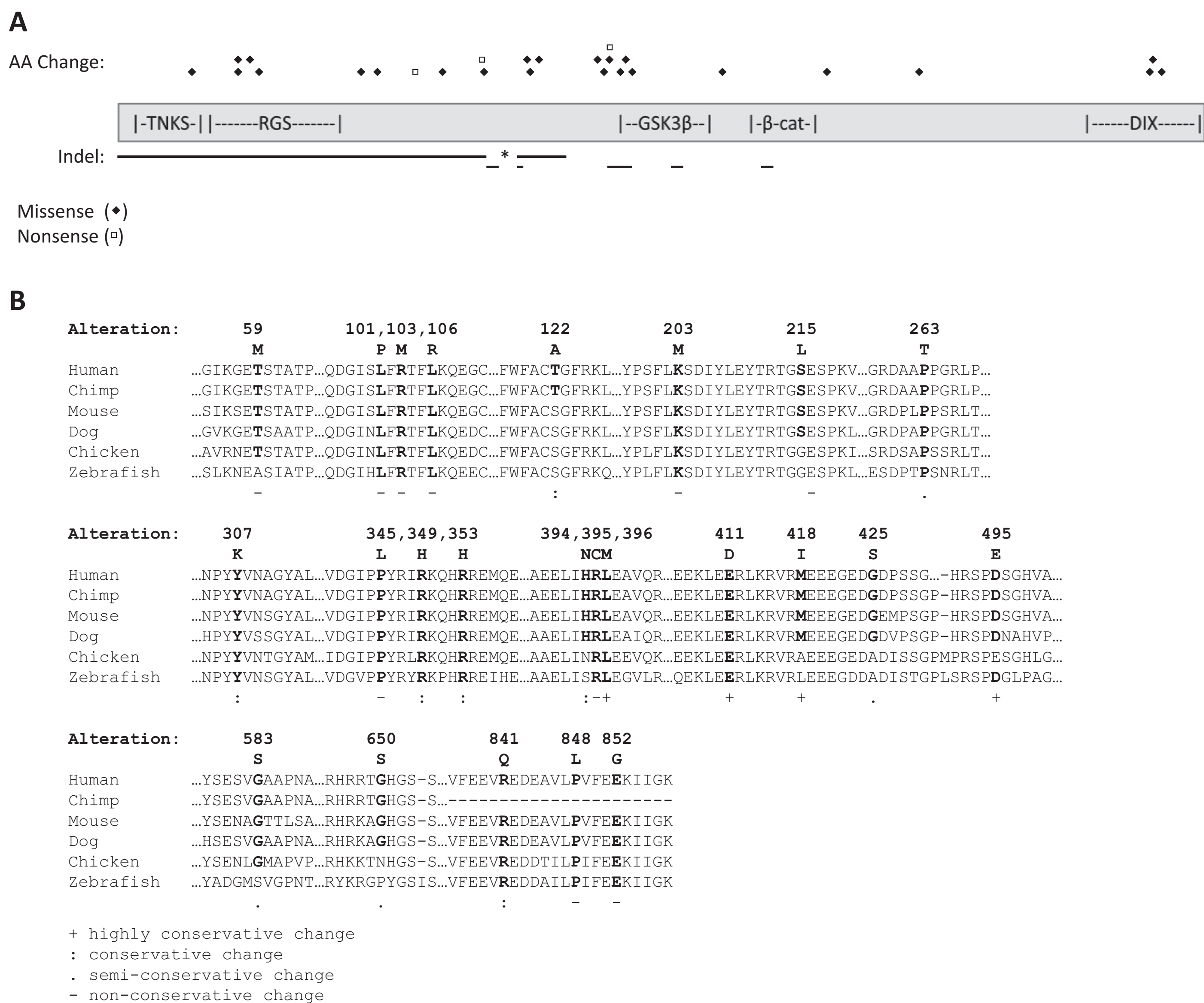


Fig. 2. Location and amino acid conservation of *AXIN1* sequence alterations reported in association with GI cancers. (A) Diagram of the location of germline and somatic *AXIN1* variants implicated in GI cancers. The locations of important functional domains in AXIN1 are indicated. Missense and nonsense mutations are shown above the diagram, insertions and deletions are shown below. (B) Amino acid conservation of the reported missense variants. Conservation designated by Gonnet [56] PAM 250 scores: > 1.5 (+), 0.5 to 1.5 (:), 0.5 to –0.5 (.), <–0.5 (–).

mutations in seven tumors, all of which were MSI-H. Four of the seven tumors also had *APC* mutations, perhaps raising some uncertainties about whether the *AXIN2* frameshift mutations were functionally significant in CRC development or just a potential marker of the MMR-defective phenotype. Finally, a survey of *AXIN2* sequence alterations in sporadic CRCs and some CRC cell lines identified several silent mutations, one 12-bp in frame deletion (*del2013-2024*), and potentially pathogenic frameshift mutation (*1993del1G*), which led to a premature stop at L688X [43]. This frameshift was seen in tumors with or without *APC* mutation, but only in MSI-H cancers, as reported previously [45]. The 12-bp deletion occurred in a MSS tumor and resulted in the in-frame loss of four amino acids, including two potential phosphorylation sites [43].

A study of a Kashmiri population looking for *AXIN1/2* mutations in CRCs found two previously reported *AXIN2* single nucleotide polymorphisms and a novel missense variant A695S [53]. The three patients with this sequence variant were diagnosed at ages 50, 57, and 65 and all presented with well-differentiated tumors with LOH of the DCC region. While the authors reported using adjacent normal tissue in their analysis, this variant has since been included in dbSNP, and because it was found in 6% of the patients, it is likely a germline polymorphism.

In addition to somatic sequence changes, changes in *AXIN2* expression have been proposed as a potential contributing factor in CRC progression [48]. In a study of gene expression data, *AXIN2* expression was significantly reduced in MSI-H CRCs compared to MSS cases. Of note, the *AXIN2* gene is directly regulated by the Wnt/β-catenin pathway, and elevated *AXIN2* expression is observed in most CRCs with *APC* mutations or other canonical Wnt pathway defects. Consistent with the gene expression data, the *AXIN2* promoter was found to be hypermethylated in 10 out of 27 MSI-H samples, but in none of the MSS samples. Additionally one of the nine MSI-H CRCs with *AXIN2* promoter hypermethylation had a frameshift leading to a premature stop codon (L688X). Ectopic expression of *AXIN2* in an MSI-H CRC cell line with low levels of endogenous protein resulted in inhibition of cell growth, suggesting that AXIN2 may act as a tumor suppressor in MSI-H CRCs [48]. A second study also found *AXIN2* methylation and reduced expression in MSI-H CRCs [60]. The authors reported that *AXIN2* expression was correlated with certain clinical and pathological features of sessile serrated adenomas, a colon cancer precursor lesion distinct from typical adenomas. These studies suggest that the level of AXIN2 protein may indeed have consequences for cancer development.

Hepatocellular carcinoma (HCC) and hepatoblastoma (HB)

Germline mutations in *APC* are associated with an increased risk of HB, suggesting other components of the Wnt pathway may be important in HB tumorigenesis [61]. Sequencing of the *AXIN1* gene in 22 HBs revealed eight exon variants, seven of which were silent [62]. The eighth variant, T58M, was also found in peripheral blood, so it may be a potentially benign polymorphism. A second study of sporadic HB tumors and HB cell lines found two large, out of frame deletions (52-bp and 1624-bp) and one somatic, missense mutation (S762N) in *AXIN2* [42] that has since been added to dbSNP.

HCCs frequently display activated Wnt signaling, but often lack mutations in *CTNNB1* or *APC*; hence, the *AXIN1* gene was screened for mutations. Out of 100 primary HCCs studied, six *AXIN1* alterations were found in five tumors, including three deletions and three premature stop codons [63]. In three of the four *AXIN1*-mutant tumors that could be fully analyzed, LOH of the wild type *AXIN1* allele was seen. Out of six HCC cell lines, two were found to contain deletions in both *AXIN1* alleles. The authors then investigated the *AXIN1* alterations in HCC lines for dysregulated Wnt signaling and/or cell proliferation. One of the two *AXIN1*-mutant HCC cell lines showed increased β-catenin reporter activity, and, in all six cell lines, overexpression of *AXIN1* was growth suppressive. The authors therefore concluded that *AXIN1* may act as a tumor suppressor in HCC.

Further analysis of HCC and HB has revealed some additional alterations in *AXIN1* and *AXIN2* [52]. While no *AXIN2* variants were seen in 27 HBs, two *AXIN1* missense variants were found (G650S and R841Q). Both of these sequence variants have since been added to dbSNP with minor allele frequencies of 0.0147 and 0.0101, respectively. Even though the authors stated that they used matched normal samples to specifically identify somatic alterations, it is possible that the reported variants were just germline polymorphisms. In a study of 73 HCCs, seven somatic variants were found in *AXIN1*: five missense alterations, one 12-bp insertion, and a 1-bp deletion leading to a premature stop codon. LOH of the wild type *AXIN1* allele was seen in four of the five *AXIN1*-mutant HCCs tested, further supporting a role for *AXIN1* as a tumor suppressor in HCC. In this same collection of 73 HCCs, two *AXIN2* sequence changes were identified, both in exon 7: a missense mutation (R659W) and a 12-bp deletion (*del2013-2024*). The 12-bp deletion has since been identified in many other cancer types, and the missense allele has been included in dbSNP.

Gastric carcinoma

Because of the highly mutable mononucleotide repeats present in *AXIN2*, one study looked for exon 7 *AXIN2* alterations in gastric cancers with MSI [47]. Nine of the 60 cancers had an *AXIN2* frameshift mutation, and all of the cancers with an *AXIN2* mutation were MSI-H.

Discussion

The potential tumor suppressor function of the AXIN1 and AXIN2 proteins has long been hypothesized, based largely on their roles in the β-catenin destruction complex. However, the importance of the AXIN proteins, *in vivo*, for the inhibition of tumor development or progression is not well established, and the functional consequences of *AXIN1* or *AXIN2* mutations in cancer remain largely undefined. While many of the reported *AXIN2* mutations cluster in one region of the gene, *AXIN1* mutations are more dispersed with no clear preference for certain sequence features or functional domains. Maps of the *AXIN1* and *AXIN2* mutations discussed above are shown in Figs. 1 and 2, along with a prediction of the functional conservation for the missense alleles. Some reported *AXIN1/2* mutations can be deemed non-contributory in cancer development, based on the lack of a clear link to familial cancer predisposition or because the variants are present at similar frequencies in controls. As noted in Table 2, many of the *AXIN1* mutations reported in cancers have since been added to the dbSNP database or identified in the 1000 Genomes Project. Further studies to compare allele frequencies in control and patient populations could shed light on whether these polymorphisms have any cancer significance. Other reported mutations need to be more thoroughly tested in clinical samples, human populations, and in cell culture and animal models to determine if they are functionally significant. Studies that address the loss of both alleles of *AXIN1* or *AXIN2* versus mutation of one allele in patient samples or animal models could help to clarify if AXINs function as classic “two-hit” tumor suppressor genes, or if the mutant alleles have a dominant effect in cancer development. A recent study found that complete loss of *Axin1* has a role in predisposition to liver cancer in a mouse model [64]. Combined with *AXIN1* somatic inactivating mutations and LOH in HCCs, these data strongly support a role for *AXIN1* as a tumor suppressor in HCC.

In addition to the many *AXIN2* mutations reported in various cancer types, a few reports have linked *AXIN2* SNPs to the disease risk in breast and lung cancers [65–67]. Further efforts may support

Table 2
Summary of *AXIN1* sequence alterations reported in GI cancers.

	Sequence alteration identified*		Cancer type	Reference	Notes
Insertions/deletions	Deletion of exons 1–2		HCC cell line: SNU475	[63]	Homozygous, no transcript detected
	Deletion of exon 4		HCC cell line: Alexander	[63]	Homozygous
	13bp deletion	Codons 346–350	HCC cell line: SNU423	[63]	LOH of second allele
	5-bp deletion	Codon 386	HCC	[63]	
	13-bp deletion	Codons 444–448	HCC	[63]	
	25-bp deletion†	Codons 485–493	HCC	[63]	
	c.1076del1bp	M418X	HCC	[52]	
	c.1714ins12bp	Insert: QVHH	HCC	[52]	
Missense		T58M	HB	[62]	Germline
		L101P†	CRC	[57]	MSI CRC, heterozygous
		R103M	CRC	[57]	MSI CRC, heterozygous
		L106R	HCC	[52]	
		T122A	CRC	[57]	MSI CRC, heterozygous
		K203M‡	CRC	[58]	
		S215L‡	CRC cell line: LS513	[40]	
		P263T‡	CRC	[39]	Heterozygous
		N307K	CRC	[58]	
		P345L	HCC	[52]	
		R349H‡	CRC	[39]	Heterozygous
		R353H†	CRC	[57]	MSI CRC, heterozygous
		H394N	CRC	[58]	
		R395C	CRC	[57]	MSI CRC, heterozygous
		L396M‡	CRC, CRC cell lines: HCT-8, HCT-15, DLD-1	[39] [40],	Heterozygous [39]
		E411D	CRC	[57]	MSI CRC, heterozygous
		M418I	CRC	[57]	MSI CRC, heterozygous
		G425S‡	HCC	[52]	
		D495E‡	CRC	[39]	Heterozygous
		G583S	CRC	[57]	MSI CRC, heterozygous
		G650S‡	HB, HCC, CRC	[52] [39],	
		R841Q‡	HB, CRC	[52] [39],	Heterozygous [39]
		P848L	CRC	[58]	
		E852G	CRC	[58]	
Non-sense		W247X	HCC	[63]	
		Y305X	CRC	[58]	
		E406X†	HCC	[63]	Seen in 2/100 cases

* Nucleotide and amino acid numbering modified to match modern sequences: NM_003502.3, NP_003493.1.
† These two mutations were found in the same individual, References [63] and [57] respectively.
‡ These variants are now listed in dbSNP (http://www.ncbi.nlm.nih.gov/SNP).

the role of *AXIN2* variant alleles in cancer predisposition and perhaps might uncover some value for the variants in informing approaches for the prevention or early diagnosis of cancer. The vast majority of the *AXIN2* mutations in cancer have been found in exon 7 and many are frameshifts, as highlighted in Fig. 1. Because the frameshift mutations are present in highly mutable mononucleotide tracts and because the mutations arise in MMR-defective CRCs, it is difficult to determine whether these frameshifts, which lead to premature stop codons, play a role in cancer development or progression, or whether they are passenger mutations. The association with a major tooth phenotype and a very strong predisposition to colon and perhaps other cancers in some individuals carrying heterozygous, germline, truncating *AXIN2* mutations strongly suggests that these mutant alleles may have major contributing roles in development and in cancer predisposition. Because the tooth phenotype is constitutional, it could inform on the function of these germline, truncating mutations. However, the role of the Wnt pathway in tooth development is complex and the tooth defect in these two families affects the development of a subset of secondary teeth, which cannot be easily modeled in the mouse. Nonetheless, it is interesting to note that some FAP patients (carrying heterozygous *APC* germline mutations) will develop extra teeth and benign odontomas [68]. If we assume that the *APC* mutations lead to activation of Wnt signaling, then the reduction in teeth in *AXIN2* mutation carriers suggests that the *AXIN2* mutant alleles might not be simple loss of function alleles, or perhaps AXIN2 plays an important role outside the β-catenin destruction complex. Further studies of the function of the wild type and mutant AXIN2 proteins may yield important clues into AXIN2 function in development and cancer.

If the AXIN proteins are indeed important suppressors of cancer development or progression, then small molecules that stabilize these proteins, such as the tankyrase inhibitor XAV939, could be valuable treatment strategies [25]. While some findings suggest that increased levels of AXIN proteins inhibit colon cancer cells that are dependent on β-catenin signaling, a recent mouse study suggests the opposite: that high levels of AXIN2 actually promote cancer cell invasion and perhaps metastasis [69]. Additionally, the AXIN proteins have been reported to be involved in multiple non-canonical signaling pathways, including the Ras/ERK [70,71] and SAPK/JNK pathways [72], so it will also be valuable to assess if any of the reported mutations in *AXIN1/2* could promote tumorigenesis via a non-β-catenin dependent mechanism. Without clear evidence of the pathogenicity of the mutations identified to date or strong evidence from animal models, the importance of AXIN1 and AXIN2 as tumor suppressor genes or oncogenes in cancer and the potential role of the variants in non-Wnt pathway functions remain unresolved issues of keen interest.

In closing, many *AXIN1/2* sequence variants in cancer have been reported. The evidence for AXIN1 as a possible tumor suppressor gene in a subset of HCCs is generally convincing, based on sequence analyses, tissue culture work, and mouse model studies. However, evidence supporting the functional significance of *AXIN1* mutations in other human cancer types is inconclusive or lacking at this point. The rare germline *AXIN2* mutations that have been associated with oligodontia and predisposition to colon and possibly other cancers provide intriguing evidence of a role for *AXIN2* mutations in CRC development. The causal significance of the somatic *AXIN2* frameshift mutations in mononucleotide repeats seen in a

subset of MSI-H cancers is unclear as the mutations may simply reflect the MSI-H phenotype. Further functional studies are needed to define the roles of the AXIN1 and AXIN2 proteins in regulating the Wnt and other signaling pathways in context- and tissue-dependent fashions, as well as to define the means by which mutant *AXIN1* and *AXIN2* alleles may contribute to cancer development and progression via classic loss-of-function ("two-hit") mechanisms, dosage-dependent mechanisms, and potentially even dominant gain-of-function mechanisms.

Conflict of interest

The authors declare that they have no financial interests or other conflicts of interest associated with this manuscript.

Acknowledgement

SM Mazzoni is supported by DOD Award W81XWH-11-1-0147.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.canlet.2014.09.018.

References

[1] N. Cancer Genome Atlas, Comprehensive molecular characterization of human colon and rectal cancer, Nature 487 (2012) 330–337.
[2] J. Groden, A. Thliveris, W. Samowitz, M. Carlson, L. Gelbert, H. Albertsen, et al., Identification and characterization of the familial adenomatous polyposis coli gene, Cell 66 (1991) 589–600.
[3] K.W. Kinzler, M.C. Nilbert, L.K. Su, B. Vogelstein, T.M. Bryan, D.B. Levy, et al., Identification of FAP locus genes from chromosome 5q21, Science 253 (1991) 661–665.
[4] S. Munemitsu, I. Albert, B. Souza, B. Rubinfeld, P. Polakis, Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein, Proc. Natl Acad. Sci. U.S.A. 92 (1995) 3046–3050.
[5] M.J. Hart, R. de los Santos, I.N. Albert, B. Rubinfeld, P. Polakis, Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta, Curr. Biol. 8 (1998) 573–581.
[6] S. Kishida, H. Yamamoto, S. Ikeda, M. Kishida, I. Sakamoto, S. Koyama, et al., Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin, J. Biol. Chem. 273 (1998) 10823–10826.
[7] Y. Xing, W.K. Clements, D. Kimelman, W. Xu, Crystal structure of a beta-catenin/axin complex suggests a mechanism for the beta-catenin destruction complex, Genes Dev. 17 (2003) 2753–2764.
[8] J. Behrens, B.A. Jerchow, M. Wurtele, J. Grimm, C. Asbrand, R. Wirtz, et al., Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta, Science 280 (1998) 596–599.
[9] H. Yamamoto, S. Kishida, T. Uochi, S. Ikeda, S. Koyama, M. Asashima, et al., Axil, a member of the Axin family, interacts with both glycogen synthase kinase 3beta and beta-catenin and inhibits axis formation of Xenopus embryos, Mol. Cell. Biol. 18 (1998) 2867–2875.
[10] M. Peifer, L.M. Pai, M. Casey, Phosphorylation of the Drosophila adherens junction protein Armadillo: roles for wingless signal and zeste-white 3 kinase, Dev. Biol. 166 (1994) 543–556.
[11] H. Yamamoto, S. Kishida, M. Kishida, S. Ikeda, S. Takada, A. Kikuchi, Phosphorylation of axin, a Wnt signal negative regulator, by glycogen synthase kinase-3beta regulates its stability, J. Biol. Chem. 274 (1999) 10681–10684.
[12] P.J. Morin, A.B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, et al., Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC, Science 275 (1997) 1787–1790.
[13] B. Rubinfeld, P. Robbins, M. El-Gamil, I. Albert, E. Porfiri, P. Polakis, Stabilization of beta-catenin by genetic defects in melanoma cell lines, Science 275 (1997) 1790–1792.
[14] G.R. Abecasis, A. Auton, L.D. Brooks, M.A. DePristo, R.M. Durbin, R.E. Handsaker, et al., An integrated map of genetic variation from 1,092 human genomes, Nature 491 (2012) 56–65.
[15] J.N. Anastas, R.T. Moon, WNT signalling pathways as therapeutic targets in cancer, Nat. Rev. Cancer 13 (2013) 11–26.
[16] J. Papkoff, B. Rubinfeld, B. Schryver, P. Polakis, Wnt-1 regulates free pools of catenins and stabilizes APC-catenin complexes, Mol. Cell. Biol. 16 (1996) 2128–2134.
[17] S. Amit, A. Hatzubai, Y. Birman, J.S. Andersen, E. Ben-Shushan, M. Mann, et al., Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway, Genes Dev. 16 (2002) 1066–1076.
[18] C. Yost, M. Torres, J.R. Miller, E. Huang, D. Kimelman, R.T. Moon, The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in Xenopus embryos by glycogen synthase kinase 3, Genes Dev. 10 (1996) 1443–1454.
[19] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, beta-catenin is a target for the ubiquitin-proteasome pathway, EMBO J. 16 (1997) 3797–3804.
[20] O. Huber, R. Korn, J. McLaughlin, M. Ohsugi, B.G. Herrmann, R. Kemler, Nuclear localization of beta-catenin by interaction with transcription factor LEF-1, Mech. Dev. 59 (1996) 3–10.
[21] M. Molenaar, M. van de Wetering, M. Oosterwegel, J. Peterson-Maduro, S. Godsave, V. Korinek, et al., XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos, Cell 86 (1996) 391–399.
[22] M. Peifer, D. Sweeton, M. Casey, E. Wieschaus, wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo, Development 120 (1994) 369–380.
[23] T.J. Vasicek, L. Zeng, X.J. Guan, T. Zhang, F. Costantini, S.M. Tilghman, Two dominant mutations in the mouse fused gene are the result of transposon insertions, Genetics 147 (1997) 777–786.
[24] L. Zeng, F. Fagotto, T. Zhang, W. Hsu, T.J. Vasicek, W.L. Perry 3rd, et al., The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation, Cell 90 (1997) 181–192.
[25] S.M. Huang, Y.M. Mishina, S. Liu, A. Cheung, F. Stegmeier, G.A. Michaud, et al., Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling, Nature 461 (2009) 614–620.
[26] F. Fagotto, E. Jho, L. Zeng, T. Kurth, T. Joos, C. Kaufmann, et al., Domains of axin involved in protein-protein interactions, Wnt pathway inhibition, and intracellular localization, J. Cell Biol. 145 (1999) 741–756.
[27] S. Ikeda, S. Kishida, H. Yamamoto, H. Murai, S. Koyama, A. Kikuchi, Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin, EMBO J. 17 (1998) 1371–1384.
[28] S. Kishida, H. Yamamoto, S. Hino, S. Ikeda, M. Kishida, A. Kikuchi, DIX domains of Dvl and axin are necessary for protein interactions and their ability to regulate beta-catenin stability, Mol. Cell. Biol. 19 (1999) 4414–4422.
[29] C. Sakanaka, L.T. Williams, Functional domains of axin. Importance of the C terminus as an oligomerization domain, J. Biol. Chem. 274 (1999) 14090–14093.
[30] I.V. Chia, F. Costantini, Mouse axin and axin2/conductin proteins are functionally equivalent in vivo, Mol. Cell. Biol. 25 (2005) 4371–4376.
[31] S. Gluecksohn-Schoenheimer, The effects of a lethal mutation responsible for duplications and twinning in mouse embryos, J. Exp. Zool. 110 (1949) 47–76.
[32] W.L. Perry 3rd, T.J. Vasicek, J.J. Lee, J.M. Rossi, L. Zeng, T. Zhang, et al., Phenotypic and molecular analysis of a transgenic insertional allele of the mouse Fused locus, Genetics 141 (1995) 321–332.
[33] H.M. Yu, B. Jerchow, T.J. Sheu, B. Liu, F. Costantini, J.E. Puzas, et al., The role of Axin2 in calvarial morphogenesis and craniosynostosis, Development 132 (2005) 1995–2005.
[34] A. Aulehla, C. Wehrle, B. Brand-Saberi, R. Kemler, A. Gossler, B. Kanzler, et al., Wnt3a plays a major role in the segmentation clock controlling somitogenesis, Dev. Cell 4 (2003) 395–406.
[35] E.H. Jho, T. Zhang, C. Domon, C.K. Joo, J.N. Freund, F. Costantini, Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway, Mol. Cell. Biol. 22 (2002) 1172–1183.
[36] J.Y. Leung, F.T. Kolligs, R. Wu, Y. Zhai, R. Kuick, S. Hanash, et al., Activation of AXIN2 expression by beta-catenin-T cell factor. A feedback repressor pathway regulating Wnt signaling, J. Biol. Chem. 277 (2002) 21657–21665.
[37] E. Lee, A. Salic, R. Kruger, R. Heinrich, M.W. Kirschner, The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway, PLoS Biol. 1 (2003) E10.
[38] L. Lipton, I. Tomlinson, The genetics of FAP and FAP-like syndromes, Fam. Cancer 5 (2006) 221–226.
[39] N.S. Fearnhead, J.L. Wilding, B. Winney, S. Tonks, S. Bartlett, D.C. Bicknell, et al., Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas, Proc. Natl Acad. Sci. U.S.A. 101 (2004) 15992–15997.
[40] M.T. Webster, M. Rozycka, E. Sara, E. Davis, M. Smalley, N. Young, et al., 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 (2000) 443–453.
[41] M.J. Smalley, E. Sara, H. Paterson, S. Naylor, D. Cook, H. Jayatilake, et al., Interaction of axin and Dvl-2 proteins regulates Dvl-2-stimulated TCF-dependent transcription, EMBO J. 18 (1999) 2823–2835.
[42] A. Koch, N. Weber, A. Waha, W. Hartmann, D. Denkhaus, J. Behrens, et al., Mutations and elevated transcriptional activity of conductin (AXIN2) in hepatoblastomas, J. Pathol. 204 (2004) 546–554.
[43] N. Suraweera, J. Robinson, E. Volikos, T. Guenther, I. Talbot, I. Tomlinson, et al., Mutations within Wnt pathway genes in sporadic colorectal cancers and cell lines, Int. J. Cancer 119 (2006) 1837–1842.
[44] L. Thorstensen, G.E. Lind, T. Lovig, C.B. Diep, G.I. Meling, T.O. Rognum, et al., Genetic and epigenetic changes of components affecting the WNT pathway in colorectal carcinomas stratified by microsatellite instability, Neoplasia 7 (2005) 99–108.

[45] W. Liu, X. Dong, M. Mai, R.S. Seelan, K. Taniguchi, K.K. Krishnadath, et al., Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling, Nat. Genet. 26 (2000) 146–147.
[46] E. Domingo, E. Espin, M. Armengol, C. Oliveira, M. Pinto, A. Duval, et al., Activated BRAF targets proximal colon tumors with mismatch repair deficiency and MLH1 inactivation, Genes Chromosomes Cancer 39 (2004) 138–142.
[47] M.S. Kim, S.S. Kim, C.H. Ahn, N.J. Yoo, S.H. Lee, Frameshift mutations of Wnt pathway genes AXIN2 and TCF7L2 in gastric carcinomas with high microsatellite instability, Hum. Pathol. 40 (2009) 58–64.
[48] K. Koinuma, Y. Yamashita, W. Liu, H. Hatanaka, K. Kurashina, T. Wada, et al., Epigenetic silencing of AXIN2 in colorectal carcinoma with microsatellite instability, Oncogene 25 (2006) 139–146.
[49] P. Peterlongo, L.R. Howe, P. Radice, P. Sala, Y.J. Hong, S.I. Hong, et al., Germline mutations of AXIN2 are not associated with nonsyndromic colorectal cancer, Hum. Mutat. 25 (2005) 498–500.
[50] S. Lejeune, F. Guillemot, J.P. Triboulet, S. Cattan, C. Mouton, P. Group, et al., Low frequency of AXIN2 mutations and high frequency of MUTYH mutations in patients with multiple polyposis, Hum. Mutat. 27 (2006) 1064.
[51] B. Rivera, J. Perea, E. Sanchez, M. Villapun, E. Sanchez-Tome, F. Mercadillo, et al., A novel AXIN2 germline variant associated with attenuated FAP without signs of oligondontia or ectodermal dysplasia, Eur. J. Hum. Genet. 22 (2013) 423–426.
[52] K. Taniguchi, L.R. Roberts, I.N. Aderca, X. Dong, C. Qian, L.M. Murphy, et al., Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas, Oncogene 21 (2002) 4863–4871.
[53] N.P. Khan, A.A. Pandith, M.U. Hussain, A. Yousuf, M.S. Khan, K.A. Wani, et al., Novelty of Axin 2 and lack of Axin 1 gene mutation in colorectal cancer: a study in Kashmiri population, Mol. Cell. Biochem. 355 (2011) 149–155.
[54] L. Lammi, S. Arte, M. Somer, H. Jarvinen, P. Lahermo, I. Thesleff, et al., Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer, Am. J. Hum. Genet. 74 (2004) 1043–1050.
[55] M.L. Marvin, S.M. Mazzoni, C.M. Herron, S. Edwards, S.B. Gruber, E.M. Petty, AXIN2-associated autosomal dominant ectodermal dysplasia and neoplastic syndrome, Am. J. Med. Genet. A 155A (2011) 898–902.
[56] G.H. Gonnet, M.A. Cohen, S.A. Benner, Exhaustive matching of the entire protein sequence database, Science 256 (1992) 1443–1445.
[57] Y. Shimizu, S. Ikeda, M. Fujimori, S. Kodama, M. Nakahara, M. Okajima, et al., Frequent alterations in the Wnt signaling pathway in colorectal cancer with microsatellite instability, Genes Chromosomes Cancer 33 (2002) 73–81.
[58] L.H. Jin, Q.J. Shao, W. Luo, Z.Y. Ye, Q. Li, S.C. Lin, Detection of point mutations of the Axin1 gene in colorectal cancers, Int. J. Cancer 107 (2003) 696–699.
[59] K. Matsuzaki, G. Deng, H. Tanaka, S. Kakar, S. Miura, Y.S. Kim, The relationship between global methylation level, loss of heterozygosity, and microsatellite instability in sporadic colorectal cancer, Clin. Cancer Res. 11 (2005) 8564–8569.
[60] Y. Muto, T. Maeda, K. Suzuki, T. Kato, F. Watanabe, H. Kamiyama, et al., DNA methylation alterations of AXIN2 in serrated adenomas and colon carcinomas with microsatellite instability, BMC Cancer 14 (2014) 466.
[61] J.E. Kingston, G.J. Draper, J.R. Mann, Hepatoblastoma and polyposis coli, Lancet 1 (1982) 457.
[62] J. Miao, T. Kusafuka, Y. Udatsu, A. Okada, Sequence variants of the Axin gene in hepatoblastoma, Hepatol Res. 25 (2003) 174–179.
[63] S. Satoh, Y. Daigo, Y. Furukawa, T. Kato, N. Miwa, T. Nishiwaki, et al., AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1, Nat. Genet. 24 (2000) 245–250.
[64] G.J. Feng, W. Cotta, X.Q. Wei, O. Poetz, R. Evans, T. Jarde, et al., Conditional disruption of Axin1 leads to development of liver tumors in mice, Gastroenterology 143 (2012) 1650–1659.
[65] H. Kanzaki, M. Ouchida, H. Hanafusa, M. Yano, H. Suzuki, M. Aoe, et al., Single nucleotide polymorphism of the AXIN2 gene is preferentially associated with human lung cancer risk in a Japanese population, Int. J. Mol. Med. 18 (2006) 279–284.
[66] J.E. Olson, X. Wang, V.S. Pankratz, Z.S. Fredericksen, C.M. Vachon, R.A. Vierkant, et al., Centrosome-related genes, genetic variation, and risk of breast cancer, Breast Cancer Res. Treat. 125 (2011) 221–228.
[67] X. Wang, E.L. Goode, Z.S. Fredericksen, R.A. Vierkant, V.S. Pankratz, W. Liu-Mares, et al., Association of genetic variation in genes implicated in the beta-catenin destruction complex with risk of breast cancer, Cancer Epidemiol. Biomarkers Prev. 17 (2008) 2101–2108.
[68] X.P. Wang, J. Fan, Molecular genetics of supernumerary tooth formation, Genesis 49 (2011) 261–277.
[69] Z.Q. Wu, T. Brabletz, E. Fearon, A.L. Willis, C.Y. Hu, X.Y. Li, et al., Canonical Wnt suppressor, Axin2, promotes colon carcinoma oncogenic activity, Proc. Natl Acad. Sci. U.S.A. 109 (2012) 11312–11317.
[70] S.H. Jeon, J.Y. Yoon, Y.N. Park, W.J. Jeong, S. Kim, E.H. Jho, et al., Axin inhibits extracellular signal-regulated kinase pathway by Ras degradation via beta-catenin, J. Biol. Chem. 282 (2007) 14482–14492.
[71] S.E. Kim, J.Y. Yoon, W.J. Jeong, S.H. Jeon, Y. Park, J.B. Yoon, et al., H-Ras is degraded by Wnt/beta-catenin signaling via beta-TrCP-mediated polyubiquitylation, J. Cell Sci. 122 (2009) 842–848.
[72] Y. Zhang, S.Y. Neo, X. Wang, J. Han, S.C. Lin, 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 (1999) 35247–35254.