Atonal bHLH transcription factor 1 is an important factor for maintaining the balance of cell proliferation and differentiation in tumorigenesis (Review)

YING FU[1*], SHA-SHA YUAN[1*], LI-JIE ZHANG[1*], ZHI-LI JI[1] and XIAO-JIANG QUAN[1,2]

[1]Key Laboratory of Diabetes Prevention and Research, Endocrinology Center, Lu He Hospital, Capital Medical University, Beijing 101149, P.R. China; [2]Laboratory of Brain Development, Institut du Cerveau et de la Moelle Épinière, Hôpital Pitié-Salpêtrière, 75013 Paris, France

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Abstract. Establishing the link between cellular processes and oncogenesis may aid the elucidation of targeted and effective therapies against tumor cell proliferation and metastasis. Previous studies have investigated the mechanisms involved in maintaining the balance between cell proliferation, differentiation and migration. There is increased interest in determining the conditions that allow cancer stem cells to differentiate as well as the identification of molecules that may serve as novel drug targets. Furthermore, the study of various genes, including transcription factors, which serve a crucial role in cellular processes, may present a promising direction for future therapy. The present review described the role of the transcription factor atonal bHLH transcription factor 1 (ATOH1) in signaling pathways in tumorigenesis, particularly in cerebellar tumor medulloblastoma and colorectal cancer, where ATOH1 serves as an oncogene or tumor suppressor, respectively. Additionally, the present review summarized the associated therapeutic interventions for these two types of tumors and discussed novel clinical targets and approaches.

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Correspondence to: Dr Xiao-Jiang Quan or Dr Zhi-Li Ji, Key Laboratory of Diabetes Prevention and Research, Endocrinology Center, Lu He Hospital, Capital Medical University, 82 XinHua South Road, Beijing 101149, P.R. China
E-mail: xiaojiang.q@gmail.com
E-mail: lhyyjzl@163.com

*Contributed equally

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1. Introduction

A balance among cell proliferation, differentiation and migration is required for proper tissue development. Epidemiological studies investigating the association between potential risk factors and an increased risk of cancer have indicated that a number of factors, including diet, obesity, hormones, immunosuppression, cancer-causing substances, chronic inflammation, infectious agents and radiation, may increase the risk of imbalance (1-3). Furthermore, genetic and/or epigenetic changes or loss of function mutations in tumorigenesis-associated genes and signaling pathways may disrupt this balance, resulting in tumor progression and metastasis (4). Previous studies investigated gene mutations of the RAS, WNT, MYC, ERK and TRK genes, that may result in tumor initiation and progression, and may assist in understanding the underlying mechanisms involved (5-8). However, little is known about the mechanism and exact cause of >100 types of human cancer. Current knowledge has revealed that only 5-10% of cancer cases have a genetic component, which indicates that gene mutation is not the sole cause of cancer development (9-14). Therefore, investigating the mechanisms underlying the alteration of protein expression profiles may aid cancer study.

Atonal bHLH transcription factor 1 (ATOH1), an evolutionarily conserved human ortholog of the *Drosophila* proneural basic helix-loop-helix (bHLH) transcription factor atonal, is involved in a variety of developmental processes. ATOH1 was cloned and identified as a proneural transcription factor based on its sequence, structure and functional features (15). ATOH1 serves an important role in the specification and regulation of skin mechanosensory cells and in the development of the auditory system in the inner ear (16,17). Furthermore, ATOH1 is required to establish the intestinal epithelium secretory cell lineage and for the development of rhombic lip derivatives, including respiratory rhythmogenesis and the cerebellar external granule cell precursor layer (15,18-20). ATOH1

positively regulates cell type specification and differentiation, controls cell cycle arrest and maintains granule neuron progenitors depending on the developmental context. Therefore, ATOH1 plays an important role in neural development and may serve as a tumor suppressor or an oncogene (21-27).

Similar to other proneural genes, including achaete-scute complex like 1 and neurogenin 2, mutations that alter the function or result in loss of function of ATOH1 are generally lethal (28). Therefore, unlike the classic oncogenes or tumor suppressor genes, ATOH1 loss of function mutations are rarely found in tumor tissue and the majority of tumors tend to exhibit abnormal increased or decreased expression of ATOH1 (21,22,26,27,29,30). Previous studies assessing the expression profile of ATOH1 in various tumor tissues revealed an alteration of ATOH1 mRNA and protein levels in brain, colon, thyroid, prostate and lung cancer (21,22,26,27,29,30). Several studies demonstrated that such alterations positively or negatively regulate tumor initiation or progression via tissue-specific mechanisms.

It is essential to identify novel molecular biomarkers for the clinical diagnosis and molecular targeting of cancer for clinical treatment. Considering the complexity of the tumorigenic progress, drug resistance, the specificity of clinical treatments and side effects, further developments are required in the field of cancer therapy. ATOH1 regulates the expression of several target genes, including BarH like homeobox 1 and hes family bHLH transcription factor 6, and influences several important signaling pathways, such as the sonic hedgehog (SHH) and notch pathways (31,32). Therefore, further investigation into the effects of ATOH1 alteration on tumorigenesis is required. The present review investigated the role of ATOH1 in cancer, with a particular emphasis on medulloblastoma (MB) and gastrointestinal cancer. Furthermore, the present review aimed to develop a clearer understanding of how alterations in ATOH1 expression and activation affect tumor initiation, progression and metastasis. Additionally, potential drug treatments for cancer therapy are discussed.

2. General features of ATOH1

ATOH1, also referred to as Hath1 in humans, Math1 in mice and Cath1 in chickens, encodes a class II bHLH transcription factor. The functional bHLH domain consists of a basic DNA-binding region and protein-binding region with two α-helices linked by a variable loop region. The protein-binding region is required for the formation of a heterodimer with a class I member of the bHLH family protein E47/E12. ATOH1 shares ~70% homology with atonal in the bHLH domain. However, the rest of the sequence exhibits much less similarity and the positioning of the bHLH domain varies among species (33,34). In vertebrates, protein sequence comparisons have revealed >80% similarity in the serine-rich region of the C-terminal (35). Additionally, the N-terminus of the open reading frame exhibits a high similarity among mammals (35). Studies on atonal and its orthologs have revealed that the non-bHLH domain of the protein serves an important role; for example, the conserved serine residues are involved in post-translational modifications which affect protein function (15,36). Domain sweeping experiments have demonstrated that specific motifs and their combinations are important for proper protein function (36,37).Over the last few decades, research has focused on identifying the downstream targets of ATOH1/atonal. The majority of the target gene candidates identified are involved in transcriptional regulation, chromosomal organization and cell cycle control and are associated with Wnt, SHH, notch, transforming growth factor-β signaling and Janus kinase (JNK)/mitogen-activated protein kinase (MAPK) signaling pathways (26,38-46).

3. ATOH1 in Merkel cell carcinoma and lung cancer

ATOH1 is expressed in Merkel cell (MCs) but not in lung tissue. In vertebrates, MCs are derived from neuroendocrine cells and are located in the basal layer of the epidermis (47). Clustered MCs consist of a touch sensitive zone, which is in nervated by slowly adapting type I mechanoreceptor nerves (48). These epidermis-derived cells are required for light touch responses (48). ATOH1 is expressed in MCs during development and in adults (49,50). ATOH1 may be required for MC progenitor differentiation, but its expression is also maintained throughout development and in mature MCs (51). ATOH1 null mice exhibited a loss of type I mechanoreceptor nerve response and lacked MCs (52). ATOH1 expression in MCs is regulated by the transcription factor SRY-box transcription factor 2 (SOX2). The expression of SOX2 in MCs is controlled by the polycomb repressor complex, which exhibits histone methyltransferase activity (53), suggesting the involvement of epigenetic regulation in cell lineage development. However, to the best of the authors' knowledge, the expression of ATOH1 in lung tissue during development has not been documented.

Abnormal ATOH1 expression in Merkel cell carcinoma (MCC). MCC is a rare malignant skin cancer derived from epithelial and neuroendocrine cell differentiation that carries a very poor prognosis (54). Approximately 80% of MCC cases are polyomavirus-positive. However, the pathogenesis involved in polyomavirus-positive and negative MCC is yet to be fully elucidated (55-57). Therefore, the association between polyomavirus infection and the development of MMC remains unclear.

Loss-of-function ATOH1 mutations or epigenetic silencing via promoter methylation have been detected in a small number of MCC cases (26). However, a recent study with a larger number of MCC cases did not determine a correlation between ATOH1 expression and MCC malignancy or an association between ATOH1 mutations and MCC development (58). Interestingly, the same study revealed a significant correlation between downregulated protein expression levels of ATOH1 and MCC recurrence or mortality (58). Further studies are required to determine the signaling pathways that interact with ATOH1 to control the differentiation of epidermal progenitors into MCs and to identify novel therapeutic agents for MCC.

Abnormal expression of ATOH1 in lung cancer. Previous studies investigating ATOH1 function during development and ATOH1 expression profiles in cancer have demonstrated the tissue- and context-specific functions of ATOH1 in physiological and pathological conditions (21-23,26,59-62). Several gene expression analyses have determined that ATOH1 is expressed in small cell lung carcinoma (SCLC),

a neuroendocrine tumor (63-66). Additionally, <20% adenocarcinoma samples express ATOH1 and these tumors exhibit neuroendocrine features (63). Cytoplasmic and nuclear expression of ATOH1 has been detected in certain lung squamous cell carcinoma (SCC) samples (30). However, ATOH1 is not known to be expressed in lung tissue and has not been reported to be involved in normal lung development (63). A correlation analysis revealed that the expression of ATOH1 was inversely correlated with lung cancer growth (25). Another study revealed that the ectopic activation of ATOH1 occurred in lung cancer, resulting in a poor patient prognosis (63). However, the underlying mechanisms remain unclear and ATOH1 expression in lung cancer cells is poorly understood. The pathways linking ATOH1 and lung cancer pathogenesis have not been fully elucidated. However, the association between ATOH1 expression and neuroendocrine tumors as well as the poor prognosis of these tumors may unravel the underlying mechanisms. Future studies investigating how ATOH1 expression alters protein expression profiles in lung cancer cells are warranted and may shed light on the mechanisms maintaining the balance among cell proliferation, differentiation and migration.

4. ATOH1 in medulloblastoma

Expression of ATOH1 in the central nervous system. The mouse ato orthologues Math1/ATOH1 mRNA is detected in the dorsal neural tube and cranial nerve ganglia at E9.5 (15). During embryonic brain development, ATOH1 is expressed in the dorsal hindbrain neuro epithelium, rhombic lip and the developing cerebellum (15,67). These ATOH1-dependent neurons are required for the generation of dorsal commissural interneurons (68) and brainstem respiratory nuclei, and the development of cerebellar granule cell lineages (18,69). Unlike MCs, in which ATOH1 is expressed in both progenitor and mature cells, ATOH1 expression persists during granule cell lineage development and in cerebellar granule cell precursors, but disappears during differentiation and migration from the external granule layer (EGL) to the internal granule layer (IGL; Fig. 1A) (18). ATOH1-null mice have a smaller cerebellum compared with wild type or heterozygous mice, and lack an EGL (18). The balance between the protein activity of ATOH1 and signaling pathway activity of SHH and Notch has been demonstrated to regulate granule cell differentiation (40). These data indicated that ATOH1 serves a crucial role in the development of cerebellar granule cells.

Abnormal ATOH1 expression in MB. Since ATOH1 is required for the regulation of cerebellar granule neuron precursors during cerebellar development, it is not surprising that ATOH1 exerts a crucial effect in the malignant cerebellar tumor subgroup SHH-MB (70), which is closely associated with the origin of granule cell progenitors (71,72). In SHH-MB, ATOH1, as well as GLI family zinc finger (GLI) 1/2, MYCN proto-oncogene, bHLH transcription factor (MYCN), cyclin D1/2 (CCND1/2) and protease nexin-1 (PN-1), are highly expressed when compared with normal tissue (73,74) and serve to control the proliferation of granule cell precursors (21-23). However, the precise mechanism of ATOH1 upregulation in MB is yet to be fully elucidated. Previous studies revealed that ATOH1 was upregulated in cases of MB with loss-of-function mutations of hypermethylated in cancer 1 (HIC1) (a ZBTB transcriptional repressor) and super-activated SHH signaling (22,59,75). Furthermore, HIC1 is required for the transcriptional inhibition of ATOH1, while cerebellar granule neuron precursors (GNPs) undergo differentiation to granule neurons, migrating from the EGL to IGL (Fig. 1A) (76). Another study revealed that the phosphorylation of tyrosine 78 in ATOH1 was present only in human colorectal cancer (CRC) and not normal tissues. This phosphorylation served to both stabilize ATOH1 and increase its transcriptional activity, hence promoting MB (77).

No evidence has demonstrated the direct activation of ATOH1 by the SHH signaling pathway, although in the majority of cases, the hyper activation of the SHH signaling pathway and high levels of ATOH1 expression are observed in SHH-MB (23,59,78). Additionally, the upregulation of ATOH1 alone does not initiate MB (21), but an increased number of MB-initiating cells were observed when both ATOH1 and Gli1 were upregulated (21). Blocking ATOH1 in GNPs limited the response of pre-proliferation genes to SHH activity and accelerated differentiation (21). This indicates that the upregulation of ATOH1 and the hyper activation of SHH may synergistically interact with each in tumorigenesis. Furthermore, ATOH1 is required for the maintenance of progenitor cells in MB progression rather than MB initiation (21,59,79,80). Gene expression data analysis has revealed a strong correlation between ATOH1 gene expression and poor survival in patients with MB (81). These data suggested that ATOH1 is a tumor progression rather than a tumor initiation oncogene in the SHH subgroup of MB.

ATOH1 in the MB-associated genetic network. Hyperactive SHH induces the expression of certain downstream genes including GLI1/2, CCND1/2 and MYCN to drive the proliferation of GNPs (82-84). In addition, a high expression of ATOH1 transcriptionally induces GLI2 expression (Fig. 1B). It has been demonstrated that ATOH1 regulates the SHH signaling pathway in GNPs (22). Furthermore, the overexpression of ATOH1 in GNPs under active SHH signaling conditions accelerates MB progression, however, proliferation was decreased in the absence of SHH (21). ATOH1 may enhance GNP proliferation by activating GLI2 and indirectly increasing the activity of the SHH signaling pathway via the inhibition of the transmembrane receptor patched 1 (PTCH1; Fig. 1B) (21,59,76).

It has been reported that <25% of MB cases are associated with constantly active mutations of the SHH pathway (85). Additionally, <20% of MB cases carry a loss-of-function mutation in PTCH1 (85). Since PTCH1 and the SHH pathway have been demonstrated to have an antagonistic relationship, hyperactive SHH mutants and loss-of-function mutations in PTCH1 strongly enhanced the progression of MB (Fig. 1B) (59). The markedly increased methylation of the HIC1 promoter results in the silencing of HIC1 expression in PTCH1 heterozygous mutant mice (21,76). Furthermore, the loss of PTCH1 may cause HIC1 silencing (Fig. 1B), which in turn deregulates its function to transcriptionally inhibit ATOH1 expression, potentially causing an increased expression of ATOH1. In addition, Ptch1 not only inhibits SHH to eliminate the enhancement function of SHH in proliferation,

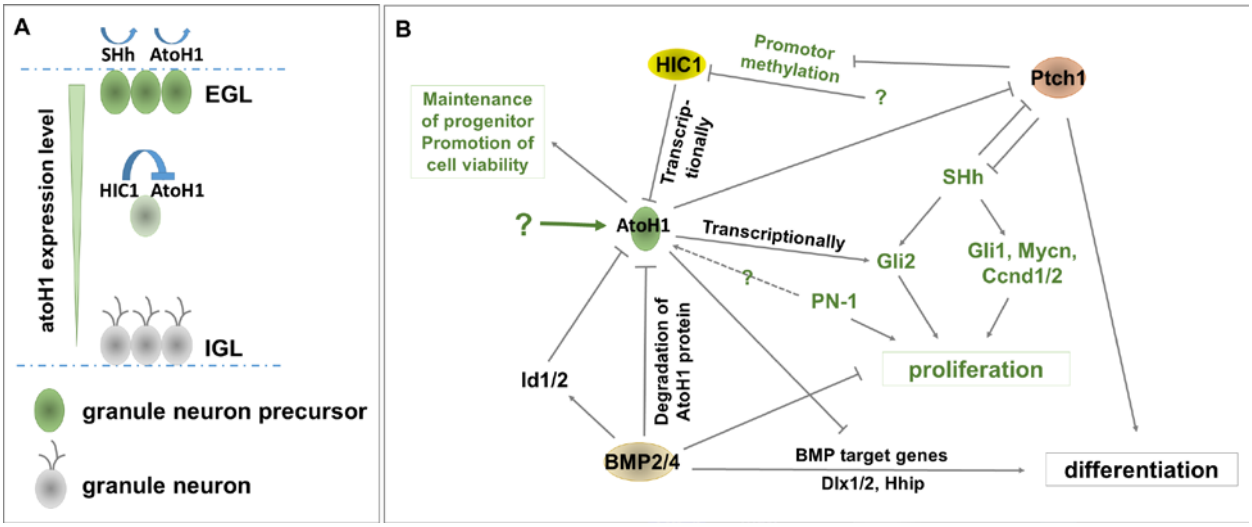

Figure 1. Schematic of granule neuron development and the genetic interactions of genes involved in medulloblastoma. (A) Diagram representing the expression and regulation of ATOH1 during granule neuron development. ATOH1 is expressed in granule neuron precursors but not in differentiated granule neurons. (B) Schematic of the medulloblastoma associated gene interaction network of ATOH1. ATOH1 promotes medulloblastoma by up regulating genes such as GLI2 to enhance proliferation and inhibit differentiation. ATOH1, atonal bHLH transcription factor 1; GLI2, GLI family zinc finger 2; SHh, sonic hedgehog; EGL, external granule layer; IGL, internal granule layer; HIC1, hypermethylated in cancer 1; Mycn, MYCN proto-oncogene bHLH transcription factor; Hhip, hedgehog interacting protein; Ccnd1/2, cyclin D1/2; Id1, inhibitor of DNA binding 1 HLH protein; Id2, inhibitor of DNA binding 2; BMP, bone morphogenetic protein; Dlx1/2, distal-less homeobox 1/2; Ptch1, patched 1; PN-1, protease nexin-1.

but also induces GNP differentiation (22). Hence, ATOH1 may repress GNP cell differentiation by downregulating PTCH1 (Fig. 1B). However, ATOH1 inhibits bone morphogenetic protein (BMP) 2/4 induced GNP cell differentiation by downregulating multiple BMP target genes. These genes, including distal-less homeobox 1/2 and hedgehog interacting protein, are required for GNP cell differentiation (Fig. 1B) (86-88).

Previous studies have revealed that ATOH1 expression can be transcriptionally inhibited by HIC1 and post-transcriptionally downregulated by BMP2/4 (79). Through the rapid proteasome-mediated protein degradation of ATOH1, BMP2/4 is able to inhibit MB progression (Fig. 1B) (21,79). However, BMP2/4 may also inhibit ATOH1 by activating the ATOH1 inhibitors inhibitor of DNA binding 1 HLH protein (ID1) and inhibitor of DNA binding 2 (ID2) (79,89). ID1/2 are transcriptional repressors, which interact with ATOH1 to prevent ATOH1-DNA binding (79,90). BMPs, a subgroup of the transforming growth factor-β superfamily of proteins, act as MB suppressors not only via the downregulation of ATOH1 expression, but also via its regulatory effect on cell differentiation and proliferation. It has been revealed that BMPs antagonize SHH-dependent proliferation by activating Smad phosphorylation and downregulating SHH-induced genes that are required for the proliferation of GNPs in MB. *In vitro* and *in vivo* data have also revealed that BMPs promote the differentiation of GNPs in MB by increasing the expression of multiple genes for cell differentiation (79).

Several genome-wide studies on gene expression profile assessing the up and downregulation of ATOH1 in MB GNPs have revealed that the outcome candidates are primarily involved in two biological processes: Cell differentiation and proliferation. Among these ATOH1 related genes, nearly two thirds are involved in differentiation and less than one third are associated with cell proliferation (79,91). Others are associated with cell adhesion, cell migration, metabolism and chromosome modulation (21,92-94). A comparative study on the categorical differences in upregulated and down regulated gene expression between the two MB groups induced by the overexpression of ATOH1 or GLI1 revealed that genes involved in neuronal differentiation, migration and adhesion, were significantly enriched in ATOH1 overexpressing MB. However, genes that regulated cell cycle progression were similarly expressed both MB groups (21). The results indicate that ATOH1 has more profound effects on controlling neuronal differentiation to promote the viability of GNPs and maintain the progenitor.

MB therapeutic intervention. As an aggressive embryonic cerebellum tumor, MB is the most common malignant pediatric brain tumor that exhibits a high mortality. The continued analysis of the mechanism that genetically and epigenetically regulates the relative gene expression of MB has permitted a deeper elucidation of therapeutic targets. The success of using Smoothened inhibitors, cyclopamine (a plant steroid alkaloid) and HhAntag (a benzimidazole derivative) as therapeutic drugs, has revealed their important regressional effect on controlling the SHH signaling pathway in MB (95,96). These agents not only decrease the proliferation of tumor cells, but also induce apoptosis of MB cells (97). However, the efficiency of these treatments are limited for MB with hyperactive SHH signaling and/or a high expression of ATOH1, since ATOH1 directly activates GLI2 expression at the transcriptional level and high GLI1/2 expressions may reduce drug efficiency. Therefore, targeting certain downstream proteins, including GLI1/2, may be more efficient (98,99). Recent reports using melanoma cell lines have demonstrated that GLI inhibitor GANT61 treatment was able to repress melanoma cells (100), indicating that additional GANT61 treatment may be effective in multiple anti-MB targeted therapy.

Although the downregulation of SHH signaling activity does not affect ATOH1 expression and a high ATOH1 expression in SHH subgroup MB represents only 25-35% of all MB cases (101), the expression of ATOH1 should still be taken into account when SHH signaling is used as a therapeutic target in MB. Despite transcription factors being difficult targets for small molecule drug development, for SHH subgroup MB, ATOH1 may still serve as a good potential therapeutic target, due to its regulatory function on GNPs in MB.

Epigenetic therapy, which reverses DNA promoter methylation in clinical treatment of myelodysplastic syndrome, has been reported (21,96). This method may be utilized to restore Hic1 function by demethylating the Hic1 promoter, thereby blocking ATOH1 expression. Another possible way to downregulate ATOH1 is via BMPs treatment. It has been demonstrated that BMPs reduce GNP proliferation and promote differentiation, as well as induce apoptosis in human MB cells (102). BMPs may therefore be used in therapeutic interventions. However, high levels of ATOH1 can override the neuronal differentiation induced by BMP by inhibiting the expression of a multiple BMP target genes (21). Therefore, similar to SHH targeting therapy, ATOH1expression levels should be considered when BMPs are used as treatment for MB.

As tumors are heterogeneous entities with different causes of disease progression, previous studies have combined two or more drugs to tackle multiple targets. For example, GNP-like MB is inhibited via effective treatment with BMPs and cyclopamine (79). Although there is no clear evidence indicating the direct effect on ATOH1 by blocking PN-1, loss of function studies on PN-1 in $PTCH1^{+/-}$ mice revealed the loss of ATOH1 expression and the reduction of MB formation (Fig. 1B) (74). Therefore, it may be worthwhile to combine the smoothened frizzled class receptor inhibitor with the PN-1 inhibitor to prevent relapse due to single drug resistance. For clinic treatment, theoretical and practical caution should be taken. Recent study that has performed multivariate Cox regression analysis has proposed a multi-gene model for MB risk prediction (81). They have also provided a quantitative analysis method for the identification of molecular markers and for the evaluation of these markers on influencing clinical behavior. More effort is required to optimize the outcome of therapeutic treatment.

5. ATOH1 in gastrointestinal cancer

Expression of ATOH1 in intestinal cells. The intestinal epithelium is a self-renewing tissue that is comprised of several cell lineages (Fig. 2A). Within the intestinal epithelium, the major cell types can be classified as absorptive (colonocytes/enterocytes) or secretory (goblet, Paneth and enteroendocrine), based on their distinct genetic programs. In the intestinal epithelium of mice, ATOH1/Math1 expression is maintained throughout embryonic and adult phases (25). The expression of ATOH1 in the intestinal epithelium is essential for the specification and regulation of proliferation of the secretory cell lineage (25). The negative regulation of intestinal epithelial cell proliferation by ATOH1 and the failure to develop all types of secretory cells in ATOH1/Math1 null mice further confirms the functions of ATOH1 in the intestinal epithelium (103,104). In addition, interaction between the ATOH1 and Notch signaling pathway regulates the lineage differentiation of different types of intestinal cells. More specifically, inhibition of ATOH1 via Hes, a Notch signaling pathway downstream gene, promoted the absorptive vs. the secretory fate (Fig. 2A) (105). Therefore, ATOH1 is required in epidermal progenitors, in their progeny to specific MCs (106) and in their commitment to neuroendocrine cells (107). These results indicate that ATOH1 is involved in the general neuroendocrine differentiation of epithelial cells.

Abnormal ATOH1 expression in CRC. It is well known that ATOH1 and Notch signaling inhibit one and another, and are involved in the development of the secretory and absorptive cell lineages. It has been demonstrated in several previous studies that intestinal ATOH1 regulates cell cycle arrest and represses proliferation, hence promoting differentiation or stimulating apoptosis (26,41). A significant decrease in ATOH1 mRNA levels has been detected in ~70% of CRC cases (24,26). In addition, the Notch signaling pathway has been determined to be associated with human colon adenocarcinomas (108). In addition to inhibiting ATOH1, Notch signaling functions to maintain stem cells in an uncommitted state (Fig. 2B) (109). Active Notch1/2 and its transcriptional target HES1 have been detected in human colon adenocarcinomas and CRC cell lines and were enriched in adenomas from adenomatous polyposis coli (APC) mutant mice (27,110-113).

Previous studies have revealed that at least one copy deletion of ATOH1 is present in ~50% of tumors and ATOH1 CpG methylation has been detected in ~70% of tumors (Fig. 2B) (26). Other evidence obtained from the analysis of 48 patients with colon cancer has revealed that ATOH1 mRNA levels drop ~20-fold and goblet cell populations are markedly reduced in colon adenocarcinomas (114). These data indicate that genetic and epigenetic mechanisms may be involved in the silencing of ATOH1 expression in CRC, and that ATOH1 is required for goblet lineage development.

Not all cases of CRC exhibit a low expression of ATOH1 mRNA (24,115,116). Previous studies have reported the loss of ATOH1 protein but the presence of ATOH1 mRNA (115). In addition to the aberrant Notch signaling pathway, the proteasomal degradation of ATOH1 by glycogen synthase kinase 3 β (GSK3β) in CRC suggested a protein level regulation on ATOH1 in CRC (Fig. 2B) (117). Aberrant constitutively activated Wnt/GSK3 signaling via truncated mutations of the APC gene is observed in ~80% of CRC cases (118,119). This aberrant Wnt/GSK3β signaling induces the continuous expression of β-catenin, which promotes the proliferation of intestinal progenitors and the proteasomal degradation of the ATOH1 protein, indicating that it is the most critical trigger for colon carcinogenesis (Fig. 2B) (117). A recent structural and functional study of ATOH1 identified a highly conserved critical serine site in the second helix domain, which, when phosphorylated, results in the quick inactivation and degradation of the ATOH1 protein (120). This result further proves the existence of another layer of ATOH1 protein regulation. However, treatment with GSK inhibitors or mutating the serine residues of ATOH1to alanine on the aforementioned or multiple serine sites may stabilize the expression and activity of ATOH1 (117,120), resulting in the induction of colon cancer cell differentiation to goblet cells (117). Another previous study

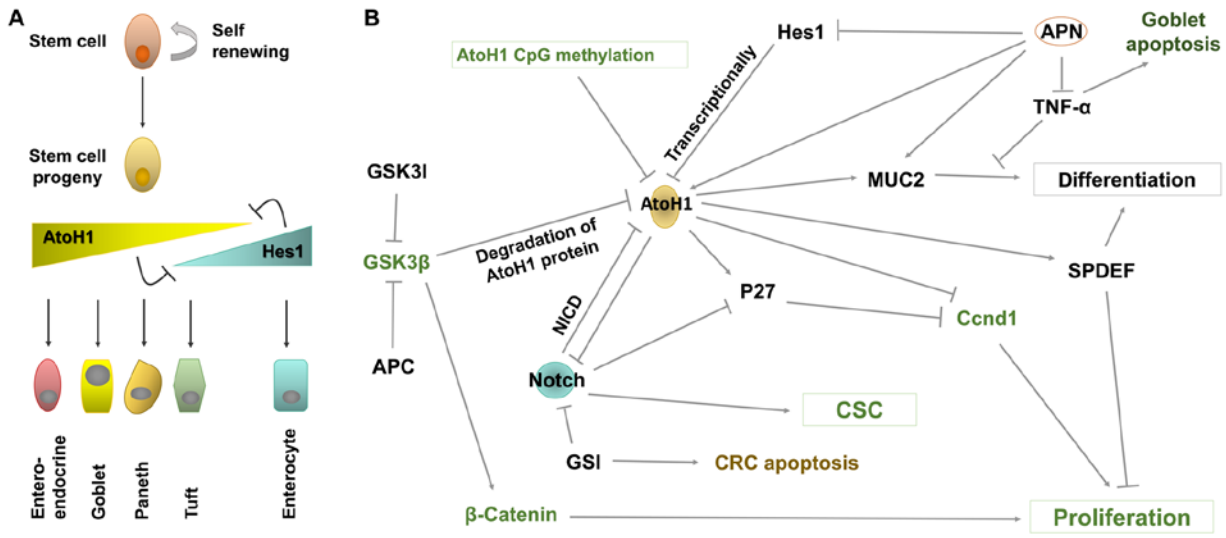

Figure 2. Schematic of intestinal epithelium cell differentiation and the genetic interactions of genes involved in CRC progression. (A) Diagram representing ATOH1 expression and regulation during the differentiation of multiple cell lineages of the intestinal epithelium. ATOH1 is necessary for the differentiation of secretory lineages in the intestine, including enteroendocrine, goblet and Paneth cells. The differentiation of different intestinal cell types is regulated by a contrary interplay between ATOH1 and Notch signaling pathways. (B) Schematic of the CRC associated gene interaction network of ATOH1. In intestinal development, ATOH1 is required for regulating target genes, which either enhances differentiation or inhibits proliferation. However, ATOH1 is downregulated by CpG methylation or GSK3β inhibition in CRC. CRC, colorectal cancer; ATOH1, atonal bHLH transcription factor 1; GSK3β, Glycogen synthase kinase 3 β; Hes1, hes family bHLH transcription factor 1; GSK3I, GSK3 inhibitor; APC, adenomatous polyposis coli; NICD, intracellular domain of the notch protein; GSI, γ-secretase inhibitor; p27, H3 histone pseudogene 23; CSC, cancer stem cells; Ccnd1; cyclin D1; APN, adiponectin; TNF-α, tumor necrosis factor-α; SPDEF, SAM pointed domain containing ETS transcription factor; MUC2, mucin 2.

also demonstrated that blocking ATOH1 protein phosphorylation promoted secretory differentiation and inhibited its involvement in the self-renewal of progenitors (121). A recent study also provided evidence that microRNA-613 promoted CRC by down regulating ATOH1 (122). The results therefore indicate that ATOH1 is down regulated in CRC.

In vitro and *in vivo* experiments have revealed that the up regulation of ATOH1 induces progenitor cell differentiation to goblet cells, inhibits proliferation and promotes apoptosis via the JNK/MAPK pathway (26,41), as indicated by the tumor suppressive nature of ATOH1 in colon adenocarcinoma and gastrointestinal carcinoma.

ATOH1 in CRC-associated genetic networks. The majority of colorectal tumors originate from epithelial cells (123). The mucus layer produced by goblet cells is part of the innate immune system and is required for intestinal homeostasis (124,125). Mucus is composed of transmembrane and secretory proteins, such as mucin (Muc) 1 and 2, respectively, that construct a semi-permeable passage between the intestinal lumen and the underlying epithelium (126,127). Previous studies have revealed that Muc2 serves as a tumor suppressor in CRC (128,129). Although wild type ATOH1 was determined to be degraded in CRC, a stable ATOH1 form or an ATOH1 phosphorylation-null mutant form alone is sufficient to induce Muc2 expression to initiate the differentiation of colon cancer cells (114,117). Apart from the direct activation of Muc2, previous studies on ATOH1 null mutant mice revealed the absence of the ETS family transcription factor SPDEF (SAM pointed domain ETS factor) and the loss of intestinal secretory cells, indicating that ATOH1 is required for the activation of SPDEF (130-132). Gain of function studies have indicated that SPDEF not only promotes the terminal differentiation of goblet/Paneth cells into goblet cells, but also inhibits the proliferation of intestinal progenitors (130-132).

The cell cycle inhibitor p27 has been determined to be activated by ATOH1 and repressed by Notch signaling via Hes1 (Fig. 2B) (114,133). ATOH1 induces cell cycle exit by inhibiting the cell cycle marker gene CCND1 either directly or via p27, blocking the proliferation of intestinal progenitors (103). ATOH1 therefore serves as a tumor suppressor, which promotes differentiation and inhibits the proliferation of intestinal progenitors.

Active Notch signaling represses p27 and ATOH1 via Hes1 activation, thereby indirectly enhancing cell proliferation and maintaining cancer stem cells (130,133,134). Similarly, the aberrant activation of Wnt/GSK3 signaling promotes cell proliferation indirectly by activating the β-catenin protein (117), whilst indirectly inhibiting cell differentiation and enhancing cell proliferation by degrading ATOH1 (115).

CRC therapeutic intervention. It has been demonstrated that a high Notch activity and an accumulation of β-catenin protein as a result of aberrant Wnt/GSK3 signaling, together with the inactivation of ATOH1, induces carcinogenesis and maintains the undifferentiated state of colon cancer (135). Therefore, treatment with GSK3 inhibitors (GSK3I), such as APC, and Notch signaling inhibitors (such as Notch-targeting antibodies or γ-secretase) may represent a novel therapeutic approach (109).

GSK is considered to be a key enzyme in various biological processes. Therefore, the development of a drug targeting Wnt/GSK signaling is difficult due to its complex protein-protein interactions and various functions in different cell types (113). The risk of treatment and pathological safety should becarefully evaluated. One particular GSK3 inhibitor,

lithium chloride, serves a protective effect against colon cancer and exerts no detectable pathological changes in other major organs while being used to treat bipolar disorder treatment (136). Previous studies assessing the molecular pathways and mechanisms involved in the cancer suppressive effect of GSK3I were performed in cell lines and rodent model systems (137-139). In addition, inactivation of the Wnt/GSK signaling pathway via the overexpression of a full-length APC gene in colon cancer cells revealed the stabilization of ATOH1 and that the degradation of β-catenin (Fig. 2B) results in cell differentiation (115). Combination with other treatments may provide a sufficient effect when compared with single GSK3I therapies for CRC (140). For instance, the overexpression of ATOH1 or the stabilization of ATOH1 protein in combination with GSK3I treatment may serve as a potential cancer therapy (135). However, detailed analyses and evaluations are required to optimize drug dosages, multiple treatments and tissue specificity. Certain cases of CRC exhibit an undifferentiated proliferative phenotype caused by constitutively activated Notch signaling (111,112,141). Maintaining a negative regulative Notch activity is initiated via the release and entry of the active domain of Notch into the nucleus. The release of the Notch signaling receptor active domain and intracellular domain requires γ-secretase activity. Theoretically, antibody-mediated Notch inhibition and γ-secretase inhibitors (GSIs) would be good candidates to inhibit colonic cancer. However, antibody-mediated Notch inhibition exerts a weak effect on blocking CRC growth (142). A previous study on γ-secretase inhibitor treatment in colon cancer cell lines and primary human CRC cell cultures revealed that GSI treatment upregulates ATOH1 expression and increases Muc2 and p27, resulting in the reduction of anchorage independent cellular growth and increasing Muc2 positive cells in ATOH1 positive CRC, but no detectable effect in ATOH1 negative CRC (134). In addition, a significant proapoptotic effect on CRC cell lines has been observed in GSI treatment (111). These data indicate that ATOH1 is crucial to the tumorigenesis regulatory network of CRC. Undifferentiated CRC represents only a fraction of colonic cancers. There are also moderately and well-differentiated classes of CRC (143). Furthermore, the hyper activation of β-catenin signaling overrides the forced differentiation induced by GSI treatment (27). These data indicate that multiple treatments or the combination of other drugs is required to obtain improved outcomes in patients.

Currently, certain cytotoxic drugs, including taxanes or platinum compounds, have been studied in CRC cell lines (144,145). However, more detailed analyses are required for further clarification. Assessing the possibility and optimizing the proper conditions of GSK3I and GSI co-treatment in CRC should be considered. Another gene, SPDEF, has been revealed to regulate the terminal differentiation of intestinal goblet cells. In breast and prostate cancer, SPDEF acts as tumor suppressor by inhibiting invasion and metastasis (26,116) and/or tumor growth and survival (24). In CRC, SPDEF serves as a key mediator of ATOH1 for tumor suppressive activity (130). Future studies are therefore worthwhile to assess whether SPDEF upregulating treatment alone or in combination with GSK3I and/or GSI is sufficient to activate goblet cell differentiation and block proliferation in ATOH1-null CRC cells. However, since ATOH1 is a potential anti-tumor gene in CRC, whether it is sufficient to block tumor growth by elevating ATOH1 levels or by force expressing ATOH1, should be elucidated. A previous study assessing the role of adiponectin (APN) in the prevention of goblet cell apoptosis and the differentiation of epithelial cells to goblet cells revealed that APN may block goblet cell apoptosis by inhibiting TNF-α and promoting differentiation by upregulating ATOH1 and Muc2, and downregulating Hes1 (74) (Fig. 2B). Therefore, APN may serve as a potential clinical target candidate for CRC.

6. Conclusions

The effect of ATOH1on the tumorigenesis of different tissue organs and the possibility of targeted clinical therapy indicated that variations in the mechanisms of tumorigenesis existed in different type of tumors. ATOH1 serves as a tumor suppressor in MCC and CRC but is an oncogene in MC and SCLC/SCC. The expression profile of the ATOH1 protein also exhibits a differential change in different types of tumors. In MCC and CRC, ATOH1 expressions are downregulated. By contrast, ATOH1 expressions were upregulated in MC and SCLC/SCC. However, regardless of these specificities, ATOH1 displayed a common feature that ATOH1 influenced tumorigenesis by promoting the transcription of its target genes for cell proliferation and differentiation. In the case of MCC and CRC, these genes are required for enhancing differentiation and inhibiting proliferation. The opposite is observed in MC and SCLS/SCC. The abnormal expression of ATOH1 results in an unpaired balance between differentiation and proliferation, which promotes the progression of cancer.

Crucial proteins are more often deregulated in multiple ways at different levels, such as at the transcriptional, translational and post translational level and during mRNA and/or protein stability. The present discussion of ATOH1 in MB and CRC indicated that the temporal/spatial regulation of protein and protein functions with tissue/context specificity are observed not only during development but also in cancer progression. Clinical drug treatments should therefore address the specificity and regulatory aspects of their targets. The cross-interaction of proteins causes single drug therapeutic treatments to be ineffective. Optimal target selection or a combined treatment approach for cancer therapy should therefore consider this cross-effect. Multiple targeted treatments have revealed a more efficient effect during cancer therapy. An improved understanding of the mechanisms of tumorigenesis and cancer regulatory networks would improve clinical approaches. The core reason for the formation of cancer is the unpaired balance of biological systems to a certain extent. Instead of assessing genes or pathways as clinical targets, understanding how this balance is disturbed and subsequently elucidating an approach for adjusting this would be another direction of cancer therapy.

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YF and SSY wrote the manuscript. LJZ wrote the manuscript and edited the figures. ZLJ and XJQ critically evaluated and revised the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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