The Somatic Mutation Hit on Top of Genetic APC mutations Cause Skin Tumor

Ting Niu*, Mingming Yang*, Qing Liu*, Haobin Li*, Lingbi Jiang*, Fanggu Li†, Xiaodong He*, Lijing Wang* and Jiangchao Li*

*Vascular Biology Research Institute, School of Life Sciences and Biopharmaceutics, Guangdong Pharmaceutical University, Guangzhou 510006, China; †The First Affiliated Hospital of Guangdong Medical University, Zhanjiang 524000, China

Abstract
Inactivation of the adenomatous polyposis coli (APC) gene is the initiating event in familial adenomatous polyposis (FAP) patients. Up to 90% of FAP patients show intestinal tumors and other extracolonic malignancies including hepatoblastomas, desmoid tumors, and brain cancer. APC mutation mice (ApcMin/+ mice) develop benign polyps in the intestinal tract. It has been reported that small numbers of ApcMin/+ mice develop breast carcinomas. Here, we found that approximately 1.6% of ApcMin/+ mice suffered skin neoplasm. The results demonstrated that these skin tumors are not derived from intestinal adenomas. Sequencing of skin tumors of ApcMin/+ mice and ApcMin/+ mice skin. The data showed that somatic mutations and gene expression levels changed greatly in skin tumors compared to control. Similarly, APC mutation accounts for 27% in the patients of nonmelanoma skin carcinomas in cancer database, and two above genes mutation coexist was observed in all patients. Furthermore, using gene mutation reagent (DMBA)—treated ApcMin/+ mice skin, the skin epithelium and glandular begin hyperplasia in ApcMin/+ mice. These findings revealed that the somatic mutation hit on the germline mutation increase the tumor incidence, suggesting that the somatic mutation should be avoided if the germline mutation exists in one body.

Translational Oncology (2020) 13, 300–307

Introduction
Inactivation of the adenomatous polyposis coli (APC) gene is the initiating event in approximately 80% of human colon cancer cases. Polyp growth is linked to the β-catenin signaling pathway, the main effector of APC mutations [1]. Familial adenomatous polyposis (FAP) is caused by an inherited mutation in the APC gene on 5p21 [2], and mutations point could occur at several different sites on this gene. Environmental factors are also thought to contribute to the variation in clinical features [3]. There is some phenotypic diversity in FAP patients, including thyroid cancer [4], hepatoblastoma [5], desmoid tumor [6], epidermoid cysts, and osteomata [7]. So, it is means that the APC mutation is observed in patients, who also frequently show simultaneous tumors at different sites, suggesting that APC is responsible for maintaining the normal epithelium growth in body. ApcMin/+ mice are an accepted model for studying intestinal spontaneous tumors with the characteristics of heritable, spontaneous, and stable adenomas, and these mice have been widely used in tumor studies, including studies of tumor growth, cardiovascular formation, apoptosis, tumor immunity, and drug therapy for tumors. Indeed, the APC gene initiates intestinal tumors in mice and leads to
the occurrence of other tumors. In addition to intestinal tumors, \(\text{Apc}^{\text{Min}+}\) mice also develop mammary tumors with low incidence (5%) [8]. Period 2 mutations also promote the incidence of intestinal tumors in \(\text{Apc}^{\text{Min}+}\) mice [9]. Recent reports indicate that treatment with the cancer drug AOM increases the risk of stomach cancer in \(\text{Apc}^{\text{Min}+}\) mice [10].

APC inactivation produces intestinal tumors, but APC inactivation is not a single reason. Intestinal inflammatory reactivations in response to the oncogenic event and the gut microbiota also contribute to the progression of this disease. High-fat diets, intestinal response to the oncogenic event and the gut microbiota also is not a single reason. Intestinal inflammatory reactivations in our laboratory, as \(\text{Apc}^{\text{Min}+}\) mice fed a high-fat diet showed more severe intestinal tumors than did mice fed normal diets (data unpublished). The newly purchased \(\text{Apc}^{\text{Min}+}\) mice had a higher incidence of tumors but pass on generations, its incidence of tumors is reduced gradually. These signs indicate that the APC inactivation is not fully responsible for the occurrence of intestinal adenoma. \(\text{Apc}^{\text{Min}+}\) mouse is not a tissue-specific gene knockout model. Adenomas in the intestinal tract are the model’s main feature. However, why nonintestinal tumors occur and what happens in this process have not been thoroughly elucidated. FAP has been reported to be associated with benign skin tumors, such as lipomas, fibroids, and epidermal cysts [11]. In this study, we found that some \(\text{Apc}^{\text{Min}+}\) mice present skin tumors, but most of the mice do not. The mice had the same environment and diet; therefore, we think that the skin tumors of these mice are caused by somatic gene mutation under the background of genetic APC inactivation. The skin cells were subjected to new somatic mutations, leading to tumor occurrence.

This study explains why some people are prone to skin tumors under the same external environment but other people did not. The mechanism behind this phenomenon may be that the genetic mutations are different for everyone. When the genetic mutation carrier encounters a new somatic mutagen, it causes tumors. Therefore, avoiding somatic mutations in external environments may decrease the cancer risk for individuals carrying genetic mutation who have a high-risk of obtaining tumors.

Materials and Methods

Mice

APC knockout mice, namely, C57BL/6J-p53 \(^{-/-}\), mice, referred to as \(\text{Apc}^{\text{Min}+}\) mice, in the C57BL/6J background were purchased from the Nanjing University Model Animal Center and were raised at the Animal Center of Guangdong Pharmaceutical University. The mouse feed was purchased from the Guangdong Medical Laboratory Animal Center and sterilized by 60 °C irradiation. Drinking water is used by ordinary urban residents and is autoclaved. The license number is SYXK (Guangdong) 2017-0125. The indoor temperature is maintained at (24 ± 2) °C, the humidity is maintained at 40%–60%, and the noise is less than 60 db. All mice received humane care and animal experiments were approved by the University Committee on the Use and Care of Animals (UCUCA) of the Guangdong Pharmaceutical University, Guangzhou, China.

Skin Tumors in \(\text{Apc}^{\text{Min}+}\) Observation and Measurement

\(\text{Apc}^{\text{Min}+}\) mice with skin tumors and control mice were sacrificed by cervical dislocation. The tumor was rinsed with PBS and placed on filter paper. The tumor tissue was photographed and the longest and short diameters of the tumor were measured. The tumor volume was calculated according to the following formula: Volume = \(\frac{4}{3}\pi ab^2\) (a is half of the long diameter and b is half of the short diameter) [12].

Genotype Identification

PCR conditions: pre-denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s, and annealing at 55 °C for 1 min; 72 °C extension for 70 s; and 72 °C extended 2 min 4 °C to cool the amplification product. The electrophoresis results of the PCR were observed in an imaging system. The amplified product length of the APC gene is approximately 340 bp (wild type band) and 600 bp (mutant band). Primer1:5'-GGCATCCCTTT CACGTTAG-3'; Primer2:5'-TTCCACTTTTGGCATAAGGC-3'; Primer3:5'-TTCTGAGAAAGACAGAAGTTA-3'.

Methylene Blue Dyeing

A methylene blue solution was prepared by dissolving 0.5 g of methylene blue powder (3,7-Bis(dimethylamino)-5-phenothiazinium chloride hydrate, A59247, Innochem) in 50 ml of absolute ethanol. After Fixing the \(\text{APC}^{\text{Min}+}\) mice small intestine with a 4% paraformaldehyde solution for 20 min, the small intestine was stained with a methylene blue solution for 30–40 s. After staining, the small intestine was differentiated using 70% ethanol for 30 s. Then, the small intestine is dedifferentiated and rinsed and the small intestine is flattened and photographed.

H&E Staining and Immunohistochemistry

H&E staining: Paraffin tissue sections were dewaxed to water at various steps, washed three times with PBS, stained with hematoxylin and eosin, washed with water after stained with hematoxylin, and sealed with neutral gum. Immunohistochemistry: Paraffin tissue sections were deparaffinized to water, washed with PBS, repaired with citrate at high temperature and high pressure, and then incubated with 3% hydrogen peroxide–methanol solution for 37 °C incubation for half an hour; 10% BSA was used for blocking. Primary antibodies ki67 (1:1000, abcam, US), CA19-9 (Clone number:121SLE, Gene Tech), Cytokeratin HMW (Clone number:34BE12, Gene Tech) were added and incubated at 4 °C overnight. The secondary antibody peroxidase-conjugated goat anti-rabbit IgG(H + L) (1:100 Item number: ZB2301, ZSGB) was further added, and DAB coloration and hematoxylin staining were performed. After dehydration, the neutral gum was used to seal the slide. Areas of interest were photographed and converted to a digital image using light microscopy equipped with camera (Olympus CX31, NY, USA).

Cancer Database Analysis

To understand the genetic mutation of APC in nonmelanoma skin tumors, we used the Cancer Database Platform (http://www. cbioportal.org/) to analyze sequencing data from the Broad Institute, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts.

The Mice Treated with DMBA

The dorsal skin of the mice was shaved three days before DMBA treatment. The C57BL/6J mice and \(\text{APC}^{\text{Min}+}\) mice were treated with 100 mg the 2,4-dimethoxybenzaldehyde (DMBA, D3254, Sigma) in 200 ml acetone under yellow light at 12 weeks old, and control mice (same number, same age of C57BL/6J mice and \(\text{APC}^{\text{Min}+}\) mice) were treated with 200 ml acetone in the same time. After 16 or 17 days,
some mice were sacrificed, the other mice were observed for survival rate.

RNA Sequencing

Tumor tissues of Apc\textsuperscript{Min/+} mice were taken and washed with 4 °C pbs, and frozen by liquid nitrogen. The RNA libraries were sequenced on the Illumina sequencing platform by Genedenovo Biotechnology Co., Ltd (Guangzhou, China).

Statistical Methods

All data were processed by GraphPad software. The t-test was used to determine whether there were significant differences among the groups. \( P < 0.05 \) was statistically significant.

Results

Abnormal Pathological Tissue Appear in Organ of Apc\textsuperscript{Min/+} Mice

Familial adenomatous polyposis (FAP) is a precancerous lesion of colorectal cancer. APC-\( \beta \)-catenin-TCF caused tumor development, as shown in Figure 1A. The Apc\textsuperscript{Min/+} mouse is an accepted model for studying intestinal tumor mice. In our study, after genotyping by PCR (Figure 1B), next observed some intestinal adenomas in the mouse intestinal tract (Figure 1C), and we compared the pathological changes in the organs of Apc\textsuperscript{Min/+} mice to control mice with H&E staining (Figure 1D). The results show that Apc\textsuperscript{Min/+} mice have slight changes and present some abnormalities. This result indicates that APC mutations can also cause slight changes in different organs of the mice, including the liver, spleen, lung, and kidney. Some red and white pulp structures disappear in the spleen and are slightly thickened on the alveolar interstitium. The glomerulus is slightly atrophic, and a number of organs show hyperplasia.

Sporadic Apc\textsuperscript{Min/+} Mice Would Occur Skin Tumors

Here, we found sporadic Apc\textsuperscript{Min/+} mice with skin neoplasms, a tumor mass of approximately 500—800 mm\(^3\). The neoplasm incidence in the mice was approximately 1.6% (6/375) (Figure 2A) and skin tissue genotype was identified with PCR (Figure 2B). The time of skin tumor phenomenon in Apc\textsuperscript{Min/+} mice is irregular. Four-week-old APC mice were found to grow the skin tumor. Twenty-four—week-old mice were also found to grow the skin tumor. And Apc\textsuperscript{Min/+} mice with skin tumors are not single sex to investigate that these neoplasms are not skin cysts, H&E staining was performed. The results showed that they are subcutaneous tumors, typical squamous cell carcinomas with cell cones (Figure 2C). Furthermore, IHC was performed with a specific antibody. The large number of keratin pearls were present in the tumor, and the tumor present positive for Ki67 antibody (Figure 2E), all suggesting that it is a squamous epithelial-derived tumor not adenocarcinoma.

Figure 1. Apc\textsuperscript{Min/+} mice present abnormal pathological characteristics in organ tissue. A. The schematic of the Wnt-Apc-tcf signaling pathway. B. The genotype of Apc\textsuperscript{Min/+} mice was identified by PCR; PCR product sizes of 340 bp and 600 bp indicated APC-mutant mice, #4, #5, and #6. The size of 600 bp indicated wild type mice, #1, #2, and #3. C. Intestinal adenoma from an Apc\textsuperscript{Min/+} mouse, shown with an arrow in schematic. D. The organs of Apc\textsuperscript{Min/+} mice were compared with those of wild type mice, including liver, spleen, lung, and kidney.
Furthermore, the CK antibody (epithelium marker) (Figure 2E) was positive, whereas CA19-9 (intestinal molecular marker) (Figure 2E) was negative. The results indicate that the tumor was not derived from intestinal tumors (adenocarcinoma). Therefore, immunohistochemical staining of these tissues indicated that this skin tumor was a squamous cell carcinoma rather than a cyst or adenocarcinoma.

These Skin Tumors Contain Somatic Gene Mutations, but Normal Skin Does Not

It has been reported that few \(Apc^{\text{Min} +}\) mice develop breast cancer; in this study, we found that skin tumors arise in \(Apc^{\text{Min} +}\) mice. Surprisingly, why most \(Apc^{\text{Min} +}\) mice do not develop skin cancer and only the 1.5% of mice develop tumors, we hypothesize that on basis of APC mutations, the skin maybe produce tumors under UV radiation or other external stimuli leading to gene mutation. To confirm this hypothesis, we sequenced the tumor tissue of \(Apc^{\text{Min} +}\) mice, with normal skin serving as a control (Figure 3A and B). The mRNA sequencing results suggest that 1507 genes are downregulated and 882 genes are upregulated significantly (Figure 3C and D). We chose a portion of the genes to show in Figure 3E, including some oncogene upregulation and tumor suppressor gene downregulation, all of which were significantly changed \((P < 0.001)\). We also present the pathway enrichment (Figure 3F), and the enrichment data show that it is notably different from that of normal skin. Additionally, the cancer signaling pathways were activated; in this study, only the Wnt pathway and PI3K were activated (Figure 3H). Finally, using SNP analysis, we found that there are some exotic gene mutations in skin tumors (Figure 3G). However, we did not determine what led to these mutations.

APC Mutations Frequently Coexist with Other Gene Mutations in Clinical Samples of Nonmelanoma Skin Tumors

Given that APC mutations frequently coexist with other gene mutations, we investigated which other mutations are present in nonmelanoma skin tumor samples from patients. Using the cancer database (http://www.cbioportal.org/) to analyze the 29 cases of DCFI, we found that nearly all skin cancer patients carried gene mutations. Most prominently, TP53 mutations accounted for 79%
of patients, and APC mutation about 21% in patients (6/21). Interestingly, we also found that APC and TP53 mutation genes are simultaneously mutated in the same patient with a frequency of 100%. In addition, there were multiple TP53 and APC mutation sites in different patients. Although these mutations occurred within these two genes, mutation points were not fixed at the same mutation sites (Figure 4C). We also note that the ratio of male to female patients and the proportion of second-generation tumors were counted (Figure 4D). Male patients were more common than female ones, and patients with both mutations also had occurred tumor metastasis.

Somatic Gene Mutation Plus Germline APC Mutation Leads to Skin Epithelium and Glandular Hyperplasia

Apc<sup>Min/+</sup> mice develop intestinal adenomas after 9 weeks because of genetic APC mutations. At the same time, few mice bearing APC mutations will develop skin tumors, we thought if some gene mutation had happened in these skin cells. The mutations in somatic cells can accelerate the frequency of tumors. To further confirm this, we use DMBA reagent to stimulate the mice skin at 12 week old, DMBA as a reagent frequently would induced gene mutation [13–15]. After 16 or 17 days, the APC mice are dead gradually but C57 mice group did not. For its histopathology, and the skin
epithelium and glandular begin hyperplasia (Figure 5C). The results showed that the mice carrying genetic mutations are more likely to develop skin tumors when somatic gene mutation hit on the top of genetic mutation (APC mutation). Summarily, the somatic gene mutation would increase the tumor incidence when germline mutation had existed in mice. It is shown in Figure 6.

Discussion

Apc<sup>Min/+</sup> mice have been widely used for more than 20 years to study the occurrence and development of intestinal tumors [16–18]. Spontaneous adenomas of the intestine are the main feature [19]. It has been reported that other organs or tissues of FAP patients also show other tumors, such as osteomas and epidermoid cysts [7,20,21]. Apc<sup>Min/+</sup> mice are whole-body systemic APC mutations; therefore, in this study, approximately 1.6% of Apc<sup>Min/+</sup> mice developed skin gland tumors. However, it is unclear why only 1.5%, not 100%, of the mice show skin tumors. We hypothesized that a point mutation in the skin epithelium caused by unexpected factors, such as ultraviolet radiation, inflammation, bites, or excessive serum fat, causes a point mutation in the repair process, leading to the occurrence of skin tumors. In this study, we confirm that these tumors are skin-derived tumors that are not metastatic from the intestinal tumor and carry...
some exotic mutations. It is suggested that the APC mutation of germline cells is a potential factor for initiating tumor formation, whereas the occurrence of somatic mutation increases the frequency of tumor occurrence. The findings of previous studies support our hypothesis [22].

We also examined the pathological features of the skin tumor to confirm that it was a skin tumor. The epithelial marker CK was used to detect that the tumor was an epithelial-derived cancer, and the intestinal tumor marker CA19-9 was not detected, indicating that the cancer was not metastatic from the intestinal adenoma.

It has been reported that gene knockout mice are used for hybridization with Apc\textsuperscript{Min/+} mice, which promotes the development of intestinal tumors. For example, we found an increase in the number of intestinal adenocarcinomas in Apc\textsuperscript{Min/+} mice, which is null for Tgfbr2 in the intestinal epithelium [23]. Smad3 mice and Apc carriers produce adenocarcinoma. The degree of tumor involvement in the MLM mice on the background of the APC mutation is more pronounced. It is suggested that APC is a necessary condition for tumorigenesis, but environmental factors affect the occurrence of tumors. In this study, we found that somatic mutations exist in skin cells, although it is not clear if these mutations drive skin tumors.

Data analysis of the cancer database showed that TP53 gene mutations in clinical patients with nonmelanoma skin tumors accounted for 80% of patients (23/29), and 6 patients with APC gene mutations were found in 29 cases of nonmelanoma. However, in APC-mutant skin cancer cases, the TP53 mutation occurred in 100% of cases. Thus, we inferred that TP53 mutation and APC mutation together promoted the occurrence of skin tumors. One study showed that Apc\textsuperscript{Min/+} mice with TP53 mutations show increased tumor rates.

Figure 5. Somatic mutation hit on APC mutations causes skin cells hyperplasia. A. The schematic diagram of the mice being treated with DMBA. B. The survival rate changes of the DMBA treated mice. The APC mutation mice survival rate becomes short. C. The results of H&E staining show that the APC mutation mice skin cells appear hyperplasia but control mice nearly normal. We can read that the hair follicle cells hyperplasia and skin epithelial cell become increased.

Figure 6. Schematic diagram of somatic mutations that increase tumor incidence when germline mutations are present in mice.
There are a number of questions that we did not answer, such as whether an increase in gene mutations in APC mice compared with normal mice following UV irradiation leads to an increased skin tumor rate. It is suggested that at least the mutation of TP53 can promote the incidence of skin tumors in Apc$^{Min/+}$ mice.

The results of this study suggest that the occurrence of skin tumors in Apc$^{Min/+}$ mice is based first on genetic mutations; next, the second somatic mutation occurs, leading to skin tumor formation. These results are significant in determining the nature of tumorigenesis, as they indicate that for normal cells to become cancer cells requires two or more gene mutations; meanwhile, some people carry genetic mutations from their parents. Somatic mutations induced by external factors could lead to the occurrence of cancer more easily, indicating that the external induction of a somatic mutation in a person with a genetic mutation increases the chance of cancer occurrence. Therefore, somatic mutations should be avoided, especially for individuals carrying genetic mutations.

**Authors’ Contributions**

Study design and conception: Ting Niu, Jiangchao Li, and Lijing Wang. Sample or data collection, analysis, and interpretation: Ting Niu, Haobin Li, Mingming Yang, Lingbi Jiang, Guanquan Mao, Qing Liu and Fenggu Li. Animal management: Ting Niu and Xiaodong He. Statistical analysis: Ting Niu and Jiangchao Li. Manuscript composition and critical revisions: Lijing Wang and Jiangchao Li.

**Conflicts of Interest**

The authors have declared that no competing financial interests exist.

**References**

[1] Dow LE, O’Rourke KP, Simon J, Tschaharganeh DF, van Es JH, Clevlers H and Lowe SW (2015). Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* **161**, 1539–1552.

[2] Waller A, Findeis S and Lee MJ (2016). Familial adenomatous polyposis. *J Pediatr Genet* **5**, 78–83.

[3] Plawski A, Banasiewicz T, Borun P, Kubaszewski L, Krokwicz P, Skrzypczal-Zielinska M and Lubinski J (2013). Familial adenomatous polyposis of the colon. *Hered Cancer Clin Pract* **11**, 15.

[4] Nagy C, Kelly Z, Keilin S, Willingham F and Chen A (2019). Barrett’s cancer screening with ultrasound in patients with familial adenomatous polyposis. *Laryngoscope* **2019**.

[5] Carr S and Kasi A (2019). Familial adenomatous polyposis. In: StatPears; 2019, Treasure Island (FL).

[6] Nefis F, Garcia L, Della Valle A, Carusso F, Vergara C, Sanchez D, Saponi M, Silveyra N, Revello AL and Espeiro P (2018). Aggressive mutation in a familial adenomatous polyposis syndrome family: when phenotype guides clinical surveillance. *J Gastrointest Oncol* **9**, 553–559.

[7] Stormorken AT, Berg T, Norum OJ, Holmebakd T, Aaerg K, Steigen SE and Grindeadal EM (2018). APC mosaicism in a young woman with desmoid type fibromatosis and familial adenomatous polyposis. *Fam Cancer* **17**, 539–543.

[8] Moser AR, Mattes EM, Dove WF, Lindstrom MJ, Haag JD and Gould MN (1993). ApcMin, a mutation in the murine Apc gene, predisposes to mammary carcinomas and focal alveolar hyperplasias. *Proc Natl Acad Sci U S A* **90**, 8977–8981.

[9] Wood PA, Yang X, Taber A, Oh EY, Ansell C, Ayers SE, Al-Assaad Z, Carnevale K, Berger FG and Pena MM, et al (2008). Period 2 mutation accelerates ApcMin/+ tumorigenesis. *Mol Cancer Res* **6**, 1786–1793.

[10] Tomita H, Yamada Y, Oyama T, Hata K, Hirose Y, Harra A, Kunisada T, Sugiyama Y, Adachi Y and Linhart H, et al (2007). Development of gastric tumors in Apc(Min/+) mice by the activation of the beta-catenin/Tcf signaling pathway. *Cancer Res* **67**, 4079–4087.

[11] Burger B, Cattani N, Trueb S, de Lorenzo R, Albertini M, Bontognali E, Itin C, Schaub N, Itin PH and Heinimann K (2011). Prevalence of skin lesions in familial adenomatous polyposis: a marker for presymptomatic diagnosis? *Oncologist* **16**, 1698–1705.

[12] Tomayko MM and Reynolds CP (1989). Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* **24**, 148–154.

[13] Zha Z, Liu T, Han F, Zhan SD and Wang CY (2015). Mutations in the p16 gene in DMBA-induced pancreatic intraepithelial neoplasia and pancreatic cancer in rats. *Hepatobiliary Pancreat Dis Int* **14**, 208–214.

[14] Osaka M, Koh T, Matsuo S and Sugiyama T (1997). The specific N-ras mutation in rat 7,12-dimethylbenz[a]anthracene (DMBA)-induced leukemia. *Leukemia* **11**(Suppl 3), 393–395.

[15] McCreery MQ, Hallwill KD, Chin D, Delrosario R, Hirst G, Vuong P, Jen KY, Hewinson J, Adams DJ and Balmain A (2015). Evolution of metastasis revealed by mutational landscapes of chemically induced skin cancers. *Nat Med* **21**, 1514–1520.

[16] Zeineldin M and Neufeld KL (2013). More than two decades of Apc modeling in rodents. *Biochim Biophys Acta* **1836**, 80–89.

[17] Cadigan KM and Nusse R (1997). Wnt signaling: a common theme in animal development. *Genes Dev* **11**, 3286–3305.

[18] Morin PJ and Weeraratna AT (2003). Wnt signaling in human cancer. *Cancer Treat Rev* **115**, 169–187.

[19] Hall E, Bercovich D and Rozen P (2009). Familial adenomatous polyposis. *Orphanet J Rare Dis* **4**, 22.

[20] Bertario L, Russo A, Sala P, Eboli M, Giarola M, D’Amico F, Gismondi V, Varesco L, Pierotti MA and Radice P, et al (2001). Genotype and phenotype factors as determinants of desmoid tumors in patients with familial adenomatous polyposis. *Int J Cancer* **95**, 102–107.

[21] de Oliveira JC, Viana DV, Zanardo C, Santos EMM, de Paula AE, Palmero EI and Rossi BM (2019). Genotype-phenotype correlation in 99 familial adenomatous polyposis patients: a prospective prevention protocol. *Cancer Med* **2019**.

[22] Levine AJ, Jenkins NA and Copeland NG (2019). The roles of initiating truncal mutations in human cancers: the order of mutations and tumor cell type matters. *Cancer Cell* **35**, 10–15.

[23] Munoz NM, Upton M, Rojas A, Washington MK, Lin L, Chytal A, Sozmen EG, Madison BB, Pozzi A and Moon RT, et al (2006). Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* **66**, 9837–9844.