Viral Oncogene Expression in the Stem/Progenitor Cell Compartment of the Mouse Intestine Induces Adenomatous Polyps.

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Conflict of interest statement: The authors declare no conflict of interest
ABSTRACT

Genetic and epigenetic events that alter gene expression and/or protein function or localization are thought to be the primary mechanism that drives tumorigenesis and governs the clinical behavior of cancers. Yet a number of studies have shown that the effects of oncogene expression or tumor suppressor ablation are highly dependent on cell-type. The molecular basis for this cell-type specificity and how it contributes to tumorigenesis are unknown. Here expression of a truncated SV40 large T-antigen in murine intestinal crypts promoted the formation of numerous adenomatous polyps in the colon and small intestine. In contrast, when the same T-antigen construct is expressed in villous enterocytes the consequences are limited to hyperplasia and dysplasia. The T antigen-induced polyps show high levels of the proto-oncogene c-Myc protein even though there is no transport of β-catenin to the nucleus. Targeting the expression of viral oncogenes to intestinal crypts or villi provides a murine model system for studying cell-type specific effects in tumorigenesis, and is particularly relevant to the study of APC/β-catenin-independent pathways contributing to the generation of intestinal polyps.

Implications: This mouse model system describes the formation of colon polyps in the absence of Wnt/β-catenin signaling.
INTRODUCTION

The functional unit of the small intestine is composed of crypts, pouch-like structures containing pluripotent stem and progenitor cells, and villi, finger-like projections above the crypts containing differentiated cells and absorbing food nutrients from the lumen. Crypt cells divide regularly, and daughter cells then migrate towards the villi, begin to differentiate and replenish the epithelium. Villi are mainly composed of absorptive cells or enterocytes (95%), with intercalated enteroendocrine and goblet cells, which provide regulatory and secretory functions respectively. Paneth cells, the fourth type of differentiated cells in the small intestine, migrate towards the bottom of the crypts and provide immune and defensive roles (1, 2).

The study of intestinal tumorigenesis has taken advantage of several mouse models, which recapitulate an/or mimic some or many aspects of the disease (3, 4). The most widely used models are based on the \(Apc^{Min}\) mouse, in which intestinal neoplasia and multiple polyps develop upon mutation of the \(Apc\) gene (5-7). Despite the central role played by the product of the retinoblastoma gene \((RB)\) in multiple cancers, current models of intestinal tumorigenesis do not address the function of \(RB\) in this disease, and the information available is somewhat controversial. For instance, an \(in\ \textit{vitro}\) system using colorectal cancer cells suggests that RB depletion would prevent proliferation and the formation of colonic tumors by preventing the degradation of \(\beta\)-catenin mediated by E2F1 (8). In contrast, several reports have indicated that removal of one or more pRb proteins from different intestinal compartments results in ectopic intestinal proliferation and hyperplasia (9-12).

In addition, our analysis of the Catalogue of Somatic Mutations in Cancer (COSMIC) obtained from the Sanger Institute [The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website, http://www.sanger.ac.uk/cosmic (13)] performed in May 2014
indicates a significant level of RB alterations present in large intestinal tumors. After excluding synonymous (silent) mutations, 101 out of 747 samples (13.5%) contained complex mutations, deletions, insertions and/or substitutions of RB. In addition, a significant percentage of large intestinal tumors show alterations in gene copy number, either in gain (272 out of 486 samples, 56%) or loss (22 out of 486 samples, 4.5%) of RB.

Finally, studies of a mouse model developed by us and others also suggest a yet uncharacterized role for pRb on intestinal cancer (14-20). Expression of the large T antigen from SV40 (SVT) in intestinal enterocytes results in hyperplasia and dysplasia and, in this system, tumorigenic induction and practically all the elicited gene expression changes depend on the ability of SVT to bind and inactivate the pRb pathway.

To test the response of intestinal stem or progenitor cells to SVT we have now used the Villin promoter to initiate expression of SVT and SVT mutants in intestinal crypts.
MATERIALS AND METHODS

Transgenic mice production

Mice expressing SV40 T antigen or the truncation mutant N136 under the control of the rat intestinal fatty acid binding protein promoter (IFABP) have been previously described (15, 21). The construction of transgenic mice expressing SVT, SVT\(^{N136}\) and SVT\(^{E107,108K}\) under the control of the villin promoter was achieved by replacing the IFABP promoter in previously described constructs with a new villin promoter. In particular, regulatory sequences from the mouse villin promoter (the 3.5Kb upstream from the transcription start site) were amplified by PCR with Pfu Ultra II (Stratagene) using specific primers which also added restriction sites (5'-CCGCGGGCGGGCCGCAATTCGACACTGTGGTGTCGCCAGCCTGGGGGA and 5'-GGTACCCTCGAGTGGTGGCAA), at 55°C annealing. The resulting 3.5Kb product was digested with Not I/Sall and ligated to the 5.4Kb NotI/SalI fragment from either IFABP.SVT; IFABP.SVT\(^{N136}\) or IFABP.SVT\(^{E107,108K}\), which contained the respective SV40 oncogenes and the plasmid backbone (21), thus effectively replacing the IFABP promoter with the villin promoter. All SVT constructs were derived from the early region of SV40 and thus can potentially express small t antigen. The mutant SVT\(^{E107,108K}\) was previously referred as TAg\(^{3213}\) (21). The 6.5 Kb fragment resulting from BamHI/HaeII digestion and containing the villin promoter and SV40 oncogene was used to microinject into pronuclei of 1-cell mouse embryos. Generation of the transgenic mice was performed at the Genetically Engineered Mouse Modeling Core at Ohio State University (http://cancer.osu.edu/research/cancerresearch/sharedresources/genetically_engineered_mouse_modeling/pages/index.aspx). Mouse genotyping was determined by 30 cycles of PCR amplification with specific primers (5'-TTTGGAGGCTTCTGGGATGC / 5'-TCCTTGGGGGCTACTGACTTCTAAGT), at 54°C annealing temperature, which generated a
537 bp product in the presence of the transgene. All lines were maintained by crosses to non transgenic FVB mice. All work involving mice was approved by both University of Pittsburgh and Ohio State University Institutional Animal Care and Use Committees and followed NIH guidelines.

**Immunohistochemical and apoptosis assays**

The mouse intestines were removed from the abdominal cavity, opened along their cephalocaudal axis, and washed with phosphate-buffered saline. Intestine was divided into proximal, middle, distal, colon and cecum sections and "swiss rolls" were prepared and fixed in formalin. Paraffin embedding followed conventional techniques and 5-µm-thick histological sections were subjected to specific antibodies to detect SV40 T antigen (PAb416); c-myc (N262 (sc-764), Santa Cruz); MMP7 (D4H5, Cell Signalling); β-catenin (BD Transduction Labs 610154) and p53 (PAb242). Antigen-antibody complexes were detected with specific biotinilated secondary antibodies followed by streptavidin-peroxidase [ABC Elite Rabbit kit (Vector Labs, Burlingame, CA); ARK kit plus Mouse on Mouse (DAKO)] according to manufacturer’s instructions. Development of the peroxidase reaction was performed with DAB substrate (DAKO). Stained sections of murine intestines were photographed under a Axio Vert.A1 Zeiss microscope.

Apoptosis was measured in 5-µm-thick histological sections by TUNEL assay following standard protocols (Research Histology Services, University of Pittsburgh).
RESULTS

*Generation of transgenic mice expressing a viral oncogene in intestinal crypts*

Expression of full length SVT in intestinal crypts appears to be extremely deleterious to the mice, as suggested by our inability to generate founder mice or transgenic progeny expressing consistently the oncogene in crypts (Table I). In fact, high expression of SVT in murine crypts was only achieved in one founder mouse, which was moribund and had to be sacrificed at 2.7 months. This mouse showed chimeric patches of expression in crypts and villi, and the expression of SVT correlated with an abnormal hyperplastic and dysplastic phenotype, villi branching, areas resembling early polyps and carcinoma (Fig. 1a-c). An associated lymphoma and carcinoma also showed partial transgene expression (data not shown), and additional anomalies were observed as well, such as liver hyperplasia and spleen enlargement.

*Role of pRb proteins in intestinal tumorigenic induction*

The retinoblastoma family of proteins comprises three closely related regulators of the cell cycle, the product of the RB gene (pRb), p107 and p130 (22). Two SVT proteins, SVT and SVT^{N136} (Fig. 1e) induce intestinal hyperplasia and dysplasia when expressed in enterocytes (19), and both are able to interfere effectively with the pRb pathway by binding the components via a functional LXCXE motif (Fig. 1e). Thus, intestinal tumorigenesis could depend on the integrity of the pRb pathway. To examine the role of pRb proteins in the induction of adenomatous polyps, we targeted the expression to intestinal crypts of a full length mutant T antigen unable to disrupt the pRb pathway (SVT^{E107,108K}; Fig. 1e). In contrast with our previous efforts to generate mice expressing SVT and SVT^{N136}, two lines of transgenic mice were readily established and
characterized containing the SVT\textsuperscript{E107,108K} transgene (Table I). Strong nuclear expression of the corresponding protein was detected from the bottom of the crypts all the way to the top of the villi along the intestinal axis (Fig. 1c), confirming that the Villin promoter efficiently directs the expression of the transgene to the intestinal crypts, and subsequently to enterocytes. The SVT\textsuperscript{E107,108K}-expressing mice developed and survived normally, had no indications of an abnormal intestinal phenotype (Fig. 1d) and showed no tumorigenesis.

In contrast, we generated 7 transgenic founders expressing the truncated SVT\textsuperscript{N136} oncogene, which retains the ability to disrupt the pRb pathway but is unable to interact with p53 (Fig. 1e). All of these founders presented a chimeric phenotype, and only some portions of the intestine showed expression of the transgene (Fig. 2b,d, Table I). The percentage of chimerism varied between founders, but transgene expression always extended from the bottom of the crypts to the tips of the villi (Fig. 2b), and higher SVT\textsuperscript{N136} levels correlated with a more evident abnormal phenotype. Transgene expression was present both in the nuclei and cytoplasm (Fig. 2d), as expected from a small protein product able to pass freely through the nuclear pores.

Unlike villi-restricted expression of SVT\textsuperscript{N136}, which results in hyper/dysplasia (Fig. 2a) (19), crypt-expression of SVT\textsuperscript{N136} produced dysplasia and also induced formation of numerous polyps along the small and large intestine (Fig. 2e). A sharp correlation was always apparent between expression of SVT\textsuperscript{N136} in particular groups of cells and/or intestinal regions and the appearance and severity of the tumorigenic phenotype, indicating that the effects of SVT\textsuperscript{N136} were cell autonomous (Fig. 2 c,d). However, our attempts to establish transgenic lines from chimeric SVT\textsuperscript{N136} founders were not successful. The founders produced either, a) no positive progeny; b) progeny presenting extremely low levels of SVT\textsuperscript{N136} expression and showing no discernible phenotype or; c)
very limited number of positive and very sick progeny (two pups) which died prior to analysis. A summary of the results is shown in Table I.

**Role of p53 and p53-dependent apoptosis in intestinal tumorigenesis**

One of the main controllers of tumorigenesis is the cellular p53 tumor suppressor, which plays roles in DNA repair, cell cycle progression and apoptosis. Disruption of the pRb pathway and other stimuli in normal cells triggers a p53-response aimed to avert abnormal proliferation. SVT is able to counteract the p53 response by directly binding, stabilizing and inhibiting p53, therefore preventing growth arrest or apoptosis and contributing to abnormal cell proliferation.

In the small intestine, we have previously shown that the expression SVT and SVT-mutants in enterocytes does not result in upregulation or disruption of p53 and p53-target genes (16, 19), and disruption of the p53 pathway is not required to induce intestinal tumorigenesis in this transgenic system (16). However, apoptosis and p53 are absent in normal intestines but, unlike in villi cells, gamma radiation triggers a robust p53 response in normal crypt cells (23). This suggests that expression of SVT and SVT mutants in crypts might affect p53 in a cell-specific manner, and prompted us to examine the levels of p53 and apoptosis in Villin-driven SVT-expressing transgenic mice. As described above, intestinal polyps were present following expression of SVT_{N136} in crypts, but not in villi (Fig.1), and we observed that, in crypts, expression of SVT_{N136} sufficed to increase the levels of apoptosis (Fig. 3a, b).

Apoptosis was restricted to the areas of SVT_{N136} expression, but was absent in normal, non-expressing areas. Expression of SVT_{N136} –which lacks a functional p53-binding domain– in crypts did not raise the levels of p53 significantly (Fig. 3d), either in areas expressing the transgene or those lacking it (Fig. 3c). In sharp contrast, expression in the crypts of SVT proteins containing a functional p53 domain binding (SVT and SVT_{E107,108K}) did raise the
levels of p53 considerably (Fig. 3f and data not shown). In this case, upregulation of – presumably inactive- p53 was found in a large subset of cells expressing the transgene, including the intestinal crypts and tumors present in the SVT founder (Fig. 3e,f and data not shown).

**Molecular characterization of SVT^{N136}-induced intestinal polyps**

We then investigated the properties of intestinal polyps in SVT^{N136}-expressing mice. Intestinal homeostasis, tumorigenesis and polyp formation have been linked to the canonical Wnt pathway and, in particular, to the nuclear translocation of β-catenin and activation of downstream target genes like c-myc (24-26) (27-31). However, mice expressing SVT^{N136} in the crypts showed a similar pattern of β-catenin localization as control mice; i.e. no nuclear β-catenin was found in either normal or abnormal intestinal areas, or in polyps or other dysplastic regions produced by the transgene (Fig. 4a). In contrast, normal nuclear localization of β-catenin in crypt cells was present in all cases (Fig. 4b).

We then monitored the expression of c-myc, a target of β-catenin frequently amplified and/or overexpressed in premalignant and malignant stages of human colorectal carcinomas (31, 32), but whose expression remains restricted to the crypt compartment in normal intestinal epithelium (33). Despite the absence of nuclear β-catenin in abnormal areas, we found that SVT^{N136} mice contained high levels of c-myc in hyperplastic villi as well as in polyps (Figure 4c,d). Areas of increased c-myc expression corresponded to areas of expression of the SVT^{N136} (Fig. 4c,f). Furthermore, the expression of MMP7 (Figure 4e), an additional β-catenin target (34), was also increased in morphologically abnormal intestinal areas expressing SVT^{N136}. Our results suggest that high levels of c-myc expression and/or other β-catenin targets can be achieved independently of nuclear β-catenin status, and that polyp formation
can be attained without nuclear localization of β-catenin.

**DISCUSSION**

We have developed a model system to induce intestinal tumorigenesis that relies on the expression of a potent oncogene, the large T antigen from SV40 (SVT), in different compartments within the intestinal epithelium. We have previously shown that the expression of SVT in the differentiated cells of the villi induces hyperplasia and dysplasia, but tumorigenesis does not proceed further under those conditions (16). Although the major cellular targets of SVT are the tumor suppressor p53 and the pRb proteins (pRb, p107 and p130), SVT induction of intestinal tumorigenesis in the villi is completely dependent on the disruption of the pRB pathway (17-19) but does not require p53 inactivation (16). SVT interferes with all three pRb proteins, therefore our results are consistent with those of other authors suggesting that the ablation of at least two members of the pRb family (pRb/p130 or pRb/p107) is required to induce ectopic proliferation in the villi (9). In contrast, we and others have shown that removal of pRb from the crypt pluripotent progenitor cells is sufficient for enterocyte proliferation (10-12). We thus aimed to understand if tumorigenic induction is dependent on which cell type the oncogene—or other alteration—is first expressed, and have generated transgenic mice that express different viral oncogenes first in intestinal crypts, and subsequently in the resulting villi.

We found that the expression of SVT in intestinal crypts appears to be extremely deleterious to the mice. In contrast, mice expressing a mutant T antigen unable to disrupt the pRb pathway (SVT^{E107,K108}) developed normally and showed no obvious phenotype,
although transgenic expression was strong in both progenitor and differentiated cells. These results indicate that SVT-mediated intestinal tumorigenesis requires disruption of the pRb pathway. Expression of the truncated SVT$^{N136}$ in the crypts was also very detrimental to the mice and we were unable to establish transgenic lines. However, 7 transgenic founders presenting a chimeric phenotype were generated and fully analyzed. Crypt-expression of SVT$^{N136}$ produced dysplasia and also induced formation of numerous polyps along the small and large intestine. These results are in clear contrast to those previously obtained upon expression of SVT$^{N136}$ restricted to intestinal villi, where mice developed hyperplasia but never showed polyp formation.

Based on our previous results (11), plus those presented in this manuscript, we propose a model to explain how Rb affects intestinal proliferation (Fig 5): The E2F activators E2F1-2-3 contribute to maintain proliferation in the crypts by activating specific promoters. As crypt cells migrate up the villi, activation of the E2F target genes is initially prevented by the binding of underphosphorylated pRb to E2F1-2-3. Final growth arrest in the villi is achieved by active repression of the promoters via p130/E2F4 (21) as part of the DREAM complex (35). In agreement with this model, cell cycle re-entry in the villi requires the loss of multiple Rb family members (9), and disruption of all pRb proteins by SVT or SVT$^{N136}$ bypasses growth restriction, allowing E2F activators to drive gene expression and induce ectopic proliferation (14, 15, 19, 21). Also in agreement with this model, expression of SVT mutants unable to disrupt the pRb pathway in the villi does not induce intestinal proliferation nor upregulation of E2F target genes (17-19), and expression in the crypts fails to induce tumorigenesis (this manuscript).

We next examined the levels of p53 and apoptosis in mice expressing SVT in intestinal crypts. Disruption of the pRb pathway induces p53-dependent apoptosis, and SVT
counteracts this response by targeting p53 directly. This binding results in p53 stabilization and cells expressing SVT contain high amounts of p53. However, p53 bound by SVT is unable to induce growth arrest or apoptosis. Although some SVT-induced tumorigenesis models (36-38) rely on p53-inactivation, expression of full length SVT antigen or an amino terminal truncation in villi neither accumulates p53 nor requires p53 to induce hyperplasia and dysplasia (16). However, and unlike enterocytes that do not show increased p53 levels or activity in response to DNA damage or T antigen expression, normal crypt progenitor cells exhibit a robust p53 response following gamma radiation (23). We thus postulated that expression of SVT and SVT mutants in crypts might affect p53 in a cell-specific manner.

We found that crypt expression of SVT\textsuperscript{N136} –which disrupts the pRb pathway but lacks a functional p53-binding domain- sufficed to increase the levels of apoptosis, which was restricted to the areas of transgene expression. However this expression did not raise the levels of p53 significantly, suggesting that either apoptosis was not mediated by p53 or that the levels of p53 were insufficient to allow for immunohistochemical detection. We believe this second possibility unlikely as, in sharp contrast, crypt expression of SVT proteins containing a functional p53 domain binding (SVT and SVT\textsuperscript{E107,108K}) did raise the levels of p53 considerably. Our results show that SVT effects on the p53 response depend on the cell type where the transgene was initially expressed and that crypt/progenitor cells are able to express p53 in response to T antigen. This study indicates that different cell types respond in a very different way to oncogenic stimuli, and that the final outcome of a particular mutation or oncogenic disruption will depend on the context -cell type, developmental and/or proliferative state- where the mutation originally took place.
What molecular mechanisms mediate the formation of intestinal polyps in SVT\textsuperscript{N136}-induced tumorigenesis? Multiple lines of evidence indicate a central role of the canonical Wnt pathway, nuclear translocation of β-catenin and activation of its downstream target genes (e.g. c-myc; (24)) in intestinal homeostasis, tumorigenesis and polyp formation (25-29). In addition, most adenocarcinomas and neoplasms of the colon show nuclear localization of β-catenin (30). Nevertheless, deregulation of target genes controlled by the β-catenin pathway was not observed in hyper/dysplastic murine intestines where the expression of SVT or SVT\textsuperscript{N136} was restricted to intestinal villi (19). However, tumorigenesis in crypt-expressing SVT\textsuperscript{N136} mice did show not only hyper/dysplasia but further progressed to polyp formation. We thus examined the status of β-catenin and some of its targets upon SVT\textsuperscript{N136} expression in the crypts. Surprisingly, we found no increase or changes in the nuclear localization of β-catenin along the intestines, including abnormal and dysplastic areas or polyps. Although this result was somewhat unexpected, a small percentage of human adenocarcinomas have no mutations on Apc or β-catenin, and is tempting to speculate that mice expressing SVT\textsuperscript{N136} in the crypts could serve as an adequate model system for those tumors. Even more unexpected was the finding that SVT\textsuperscript{N136} mice expressed high levels of c-myc, a transcriptional target of β-catenin, in hyperplastic villi and polyps. This result was particularly intriguing because but the c-myc gene is frequently amplified and/or overexpressed in premalignant and malignant stages of human colorectal carcinomas (31, 32), although c-myc expression is restricted to the crypt compartment in normal intestinal epithelium (33). Furthermore, the expression of MMP7, an additional β-catenin target (34), was also increased in intestinal areas expressing SVT\textsuperscript{N136}. We conclude that SVT\textsuperscript{N136} induces high levels of c-myc expression and possibly other β-catenin targets independently of nuclear β-catenin status, and that polyp formation can be attained without nuclear localization of β-
Other pathways, such as rapid protein degradation, can control c-myc independently of β-catenin nuclear localization (39). One possibility is that SVT^{N136} prevents c-myc degradation and thus results in its accumulation.

A subset (10-12%) of human adenocarcinomas contain no mutations of Apc or β-catenin but still show high expression levels of c-myc, indicating that regulation of c-myc in these tumors is independent of the Apc/β-catenin pathway. Our results indicate that SVT^{N136} uses alternative mechanism/s -independent of upstream regulation by Wnt- to activate β-catenin targets and contribute to the formation of polyps in the small and large intestine. Thus, SVT^{N136} expression in crypts appears to mimic the subset of colorectal tumors that lack Apc/β-catenin mutations.

At present we do not know what β-catenin-independent mechanisms induce polyp formation. Our recent work indicate that control of normal (wt crypts) and ectopic (RBKO villi) intestinal proliferation depends on a combination of two different pathways controlled by pRB, the E2F activators and c-myc (Liu et al, submitted). In agreement, induction of tumorigenesis in our model system depends on an intact LXCXE motif and, presumably, deregulation of pRb proteins. We hypothesize that disruption of pRb in the crypts by SVT^{N136} results in c-myc expression changes that contribute to polyp formation independently of β-catenin. Ectopic c-myc expression is necessary but not sufficient to induce intestinal polyps (40). Thus, our results suggest that additional cellular proteins targeted by SVT^{N136} contribute to the induction of polyps. These targets will be the focus.
of future investigations and could allow us to design specific therapies matched to genetic
background of particular adenocarcinomas.

In summary, we have found that the molecular consequences and tumorigenic phenotype
caused by T antigen expression in the intestine depend on the initial subset of cells where
the transgene is expressed. SVT$^{N136}$ only induces polyp formation when targeted to the
proliferating cells of the crypts. Expression of SVT in crypts, but not in villi, is able to
increase -and presumably, inactivate- the levels of p53. Our results indicate that
disruption of the pRb and/or p53 pathways has different consequences in different
subsets of cells and could help to explain why the role of pRb in intestinal tumorigenesis
has not been completely established. In addition, we have shown that intestinal
tumorigenesis could arise independently of β-catenin nuclear localization. Identification of
the redundant /cooperating pathways operating in this system could lead us to valuable
insights to evaluate and treat different cancers.

ACKNOWLEDGEMENTS

This work was supported by NIH grant R01CA098956 to J.M.P.
FIGURE LEGENDS

Figure 1. SVT expression in the crypts induces intestinal tumorigenesis, while an SVT mutant unable to disrupt the pRb pathway fails to produce a phenotype.

Immunohistochemical staining of intestinal murine sections with an antibody specific for SVT and mutant derivatives, indicated by brown color, reveals expression of the transgene driven by the Villin promoter. (a) Villin-driven expression of SVT initiates at the bottom of the crypts and extends towards the tips of the villi (a, detail in "c"). Expression of the transgene correlates with the appearance of abnormal intestinal areas as shown in serial sections stained for SVT (a) or H&E (b), and include one polyp (blue arrows). (d) Histological section from the proximal intestinal region of a 6 month old transgenic male carrying Villin.SVT^E107,108K. Expression of the SVT^E107,108K transgene extends from the bottom of the crypts to the tip of the villi, but nevertheless produces no discernible anomalies or tumorigenic phenotype in the mice. (e) Top: Schematic diagram of the different protein domains and motifs within SVT (in light gray: J domain, LXCXE motif, nuclear localization signal, origin binding-, Zn-, ATPase- and host range domains) and the cellular partners they bind (in dark gray: hsc70, pRb proteins, p53). Bottom: SVT mutants used in this study (Full length –SVT-, N-terminal truncation -N136-, mutant defective for pRb binding –E107,108K-).

Figure 2. Expression of an SVT mutant in the intestinal crypts leads to polyp formation.

(a-c) Histological sections from proximal regions of murine intestines showing the presence of SVT^N136 as detected with a specific antibody, indicated by brown color: While SVT^N136 transgene expression driven by the IFABP promoter (IFABP. SVT^N136) is restricted to the villi and results in hyperplasia (a), expression driven by the villin promoter (Villin.SVT^N136) initiates from the crypts and results in overt dysplasia, neoplasia and polyp formation (b-d).
of serial sections from Villin.SVTN136 mice by hematoxilin and eosin staining (e) or SVT\textsuperscript{N136} immunostaining (d) indicate a clear correlation between transgene expression and neoplastic phenotype.

**Figure 3. Apoptotic response to SVT\textsuperscript{N136} expression and induction of p53 in different intestinal compartments.** Immunohistochemical staining of serial sections from SVT\textsuperscript{N136} chimeric mice reveal that the extensive apoptosis measured by TUNEL assay (a, brown staining) correlates with the presence of the transgene (b, brown staining). Apoptosis is evident in areas of SVT\textsuperscript{N136} expression (red arrowheads) and is absent in normal areas lacking SVT\textsuperscript{N136} expression (blue arrowheads). Expression of p53 and the appropriate transgene was monitored in serial sections from SVT\textsuperscript{N136} (c, d) and SVT (e, f) mice. SVT\textsuperscript{N136} expression, even in crypts (c, blue arrows) is not able to raise and/or stabilize substantially the levels of p53 (d, arrows). In contrast, expression of full length SVT (e) raises significantly the levels of p53 (f).

**Figure 4. SVT\textsuperscript{N136} expression in crypts induces morphological changes and correlates with increased levels of c-myc without altering the localization of \( \beta \)-catenin.** The presence of different antigens [\( \beta \)-catenin (a,b); c-myc (c,d); MMP7(e) and SVTN136 (f)] was revealed by immunohistochemistry of murine intestines and is indicated by brown color. Sections from Villin.SVT\textsuperscript{N136} mice show that \( \beta \)-catenin nuclear staining is absent in dysplastic or polyp areas (a), while the normal nuclear localization is evident in intestinal crypts (b). Increased levels of c-myc protein are apparent in abnormal regions (c) and polyps (d). As shown by comparison between serial sections (c, f), the areas of c-myc expression (c) correlate to areas of SVT\textsuperscript{N136} expression (f). The presence of MMP7 in adjacent
morphologically normal and abnormal areas of Villin.SVT\textsuperscript{N136} intestines is shown in (e): While MMP7 staining is restricted to the bottom of the crypts in normal intestinal areas (red arrows), it extends up the crypts and into the villi in abnormal areas (blue arrows), which also express the SVT\textsuperscript{N136} transgene (not shown).

**Figure 5. Diagram of the villi-crypt intestinal unit and model for the control of intestinal proliferation by the Rb/E2F pathway.** Inactive hyperphosphorylated pRb allows E2F-mediated S-phase progression and proliferation in stem and progenitor cells within the crypts. As cells migrate upwards to differentiate and form the quiescent villi, transient repression of cell cycle gene transcription is first mediated by pRb binding to E2F activators in the promoters of E2F-target genes. Promoters then become permanently repressed by p130-E2F4 DREAM complexes at the final stages of differentiation and growth arrest.

**REFERENCES**

<table>
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<th>Construct</th>
<th>Transgene expression and number of mice</th>
<th>Expression details</th>
<th>Founder morphology</th>
<th>Offspring produced?</th>
<th>Positive progeny?</th>
<th>Transgene expression and morphology of progeny</th>
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<td>Villin SVT (7 founders)</td>
<td>Chimeric expression from bottom of crypts to tips of villi (2 mice)</td>
<td>High expression (1 mouse)</td>
<td>Very unhealthy, harvested at 2.7 months. Intestinal dysplasia, carcinoma and lymphoma.</td>
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<td>Yes (from one founder)</td>
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<td>Transgene expression limited to top of crypts and villi, absent from bottom crypts. Hyperdysplastic intestines</td>
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<td>Villin SVT ( ^{1136} ) (11 founders)</td>
<td>Chimeric expression from bottom of crypts to tips of villi. Both nuclear and cytoplasmic (7 mice)</td>
<td>Very high expression (1 mouse)</td>
<td>Hyperdysplastic areas, several small polyps, elongated crypts, hyperplastic Peyer patches. Abnormal areas correlating with staining.</td>
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<td>Good expression (2 mice)</td>
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<td></td>
<td>Very high expression, high percentage of tissue expressing the transgene (1 mouse)</td>
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<td>Very sick, Died prior to analysis (1.7 months)</td>
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</tr>
<tr>
<td></td>
<td>Very high expression, low percentage of tissue expressing the transgene (1 mouse)</td>
<td>Hyperdysplastic areas, some polyps</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low expression (2 mice)</td>
<td>Hyperplastic and dysplastic patches, long villi, fused crypts. One very sick mouse had liver cancer</td>
<td>Yes (from one mouse)</td>
<td>Yes</td>
<td>Very low expression from bottom of crypts. Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Undetectable (3 mice)</td>
<td>Normal</td>
<td>Yes (1 mouse)</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Died prior to analysis (1 mouse)</td>
<td>Not determined</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villin SVT ( ^{207,166} ) (4 founders)</td>
<td>Not analyzed (2 mice)</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes (from 2 founders)</td>
<td>Transgene expression from the bottom of the crypts. Normal morphology</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Undetectable (1 mouse)</td>
<td>Normal</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low/mid expression (1 mouse)</td>
<td>Normal</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table I.** Summary and details of founder mice used in this study
Viral Oncogene Expression in the Stem/Progenitor Cell Compartment of the Mouse Intestine Induces Adenomatous Polyps.

Maria Teresa Saenz Robles, Ph.D., Jean Leon Chong, Christopher Koivisto, et al.

*Mol Cancer Res* Published OnlineFirst July 3, 2014.

Updated version

Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-14-0166

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