Atm is a Negative Regulator of Intestinal Neoplasia

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Abstract

The *ATM* (Ataxia telangiectasia mutated) gene has been implicated as an early barrier to the growth and progression of incipient solid tumors. Here, we show that germline nullizygosity for the mouse *Atm* gene significantly increases the proliferative index, net growth rate, and multiplicity of intestinal adenomas in two distinct models of familial colon cancer: $Apc^{Min/+}$ and $Apc^{1638N/+}$. These effects of *Atm* deficiency are quantitatively different from deficiency for either of the genomic stability genes Bloom's Syndrome helicase (*Blm*) or DNA ligase IV (*Lig4*), and the effect of *Atm* loss on tumor multiplicity is largely independent of the effect of ionizing radiation. Furthermore, the LOH (loss of heterozygosity) rates at the *Apc* (Adenomatous polyposis coli) locus are unaffected by *Atm* loss. Taken together, these data implicate the *Atm* gene product as a barrier to dysplastic growth in the early stages of intestinal tumor progression, independent of its effects on genomic stability.

Keywords

Gastrointestinal cancer; Atm; Apc; Blm; Lig4; modifier

Action of the *ATM* gene product is highly pleiotropic; its loss of function is associated with a wide range of neurological and aging phenotypes, including a strong predisposition to lymphoid malignancies (Savitsky *et al.*, 1995). Recently, it has been discovered that the Atm pathway suppresses the progression of various solid tumors by triggering DNA damage response mechanisms, including senescence (Gorgoulis *et al.*, 2005; Bartkova *et al.*, 2005; Bartkova *et al.*, 2006). *Atm*-deficient mice rapidly develop aggressive thymic lymphomas (Barlow et al., 1996), but have not yet shown a predisposition to epithelial neoplasia. We have therefore sought to test these aspects of Atm function using mouse models for epithelial cancer. The intestine provides a powerful experimental platform to ask whether *Atm* deficiency affects the formation and progression of solid tumors on *Apc*-mutant backgrounds. $Apc^{Min/+}$ mice - which have a stop codon at position 850 - develop multiple tumors throughout the intestinal tract, and are widely used to test for the effects of various mutations and environmental factors on tumorigenesis (Taketo, 2006); $Apc^{1638N/+}$ - which truncates *Apc* at codon 1638 and decreases protein levels to ~2% (Fodde *et al.*, 1994) - is an independent model that develops 1–2 intestinal tumors with biological and molecular characteristics different from those of $Apc^{Min/+}$ tumors (Haigis *et al.*, 2004). We compare the results of crosses of *Apc*-mutant mice to *Atm*-deficient mice with similar crosses to mice deficient in the genomic stability proteins Bloom's helicase (Blm) or DNA ligase 4 (Lig4).

We introgressed a null *Atm* allele (Barlow *et al.*, 1996) onto the C57BL/6J (B6) background before crossing it to B6.$Apc^{+/-}$ mice. $Atm^{-/-}$ animals on both the B6 and 129/S6 (129) backgrounds showed a significant reduction in body mass, accompanied by a consistent shortening of the small intestinal tract by an average of 3 cm (data not shown). B6.$Atm^{-/-}$ mice are also infertile and developed thymic lymphomas at a reduced rate (10/44, 23%) compared

to age-matched 129.$Atm^{-/-}$ animals (9/14, 63%; p<0.05, Fisher's Exact Test). However, the lymphoma phenotype did not correlate with any intestinal phenotypes in $Apc^{+/-}$ derivatives of these $Atm^{-/-}$ genotypes.

To generate the three possible Atm genotypes on an Apc-mutant background, we intercrossed B6.$Atm^{+/-}$;$Apc^{+/-}$ and B6. $Atm^{+/-}$;$Apc^{+/+}$ mice. B6.$Atm^{-/-}$ mice on an $Apc^{Min/+}$ background developed 2-fold more intestinal tumors than heterozygous Atm or wildtype age-matched controls (Table 1a). To normalize for the length of the intestinal tract, tumors/cm² were measured and also found to be significantly different: $Atm^{+/+}$, 0.4 ± 0.1; $Atm^{+/-}$, 0.4 ± 0.1; $Atm^{-/-}$, 0.9 ± 0.1 (p<0.01, Kruskal-Wallis test). Atm deficiency also produced a significant increase in colon tumor multiplicity and incidence. Notably, $Atm^{+/-}$ heterozygotes did not differ significantly from $Atm^{+/+}$ homozygous wildtype mice for tumor multiplicity in the small intestine (p>0.1) or colon (p>0.2) or for incidence of colonic tumors (p>0.6). However, the average tumor diameter at 100–109 days of age increased significantly with decreasing allelic dosage of wildtype Atm (Fig. 1). The increase in size was uniform throughout the length of the intestine, with each quartile of the small intestine demonstrating a significant difference (p<0.02, Jonckheere-Terpstra test; data not shown). Preliminary data from four mice indicates that this size increase also holds true at 160–168 days of age (data not shown). We investigated whether the increase in tumor size reflected an enhancement of cellular proliferation. Immunohistochemical staining for the Ki-67 antigen revealed an increase in the percentage of Ki-67-positive cells in $Atm^{-/-}$;$Apc^{Min/+}$ tumors and in tumor-associated hyperplasia when compared to $Atm^{+/+;}$ $Apc^{Min/+}$ (Fig. 2a–f and Table 2).

We then asked whether Atm-deficiency affects a different model of intestinal tumorigenesis, $Apc^{1638N/+}$. First, we confirmed that the molecular mechanisms involved in $Apc^{1638N/+}$ tumors differed from $Apc^{Min/+}$ tumors. $Apc^{Min/+}$ tumors showed a pattern of strong nuclear β-catenin staining in Apc-negative cells - a hallmark of neoplastic $Apc^{Min/+}$ tissue. This observation was independent of the Atm status (12/14, 86% and 20/22, 91% for $Atm^{+/+}$ and $Atm^{-/-}$, respectively; Fig. 2g–1). By contrast, all 28 $Apc^{1638N/+}$-induced tumors (14 $Atm^{+/+}$ and 14 $Atm^{-/-}$) were negative or only weakly positive for nuclear β-catenin, despite the loss of detectable Apc antigen (Fig. 2, compare m–r to g–l). We controlled for differences in staining by processing $Apc^{Min/+}$ and $Apc^{1638N/+}$ tumor sections in parallel. A similar result has been reported elsewhere (Janssen et al., 2006), but it remains unclear by what alternative mechanism $Apc^{1638N/+}$ tumors initiate.

Next, we found that nullizygosity for Atm dramatically increased the multiplicity of $Apc^{1638N/+}$ tumors in the small intestine, by 16-fold (Table 1a). Atm heterozygotes did not differ from Atm wildtype mice in the multiplicity of $Apc^{1638N/+}$ tumors in the small intestine (p>0.7). Colonic tumor multiplicity and incidence were also significantly increased by Atm deficiency, as both $Atm^{+/-}$ heterozygous and $Atm^{+/+}$ wildtype control groups of $Apc^{1638N/+}$ developed no colonic tumors, while 45% of $Atm^{-/-}$ derivatives developed at least one. These colonic tumors showed a histological appearance indistinguishable from those of $Apc^{Min/+}$ animals. The low overall tumor multiplicity in controls precluded a rigorous statistical analysis of tumor size. Overall, these results indicate that loss of Atm function enhances intestinal tumorigenesis, regardless of the initial underlying molecular mechanism.

Because the functions of Atm encompass more than cellular growth, we tested the effects of Atm loss on genomic stability. First, we measured rates of LOH at the Apc locus in $Apc^{Min/+}$ mice. Quantitative Pyrosequencing® assays (Pyrosequencing is a registered trademark of Biotage) for the $Apc^{Min/+}$ SNP (performed as described in Amos-Landgraf et al., 2007) demonstrated no statistically significant difference in the percentage of LOH-initiated tumors among the three genotypes: $Atm^{+/+}$, 31/33 (94%); $Atm^{+/-}$, 29/34 (85%); and $Atm^{-/-}$, 45/51 (88%), ruling out a strong effect of Atm loss on this mechanism of tumor initiation. Second,

we measured the regional localization of intestinal tumors, which is hypothesized to correlate with the mechanism of induction (Haigis *et al.*, 2004). *Atm* deficiency did not affect the regional distribution of tumors per 10%- or 25% sub-interval of the small intestine, for either $Apc^{Min/+}$ or $Apc^{1638N/+}$ mice (data not shown). Finally, we induced DNA double-strand breaks, using whole-body ionizing radiation (IR). Unlike the acute sensitivity of $129.Atm^{-/-}$ mice to IR, $B6.Atm^{-/-}$ mice with or without *Apc* mutations survived for over two months after a single 5Gy dose at 10 days of age. Dissections of age-matched mice showed that, after IR, Atm deficiency affected the multiplicity of $Apc^{Min/+}$-induced tumors in the colon, but not in the small intestine, compared to irradiated $Atm^{+/+};ApcMin/+$ controls (Table 1a). However, after adjusting for intestinal length, a significant effect of *Atm* deficiency was seen for tumors/cm² in the small intestine: $Atm^{+/+}$, 3.3 ± 0.6; $Atm^{+/-}$, 3.4 ± 0.5; $Atm^{-/-}$ 4.4 ± 0.4 ($p<0.05$, Jonkheere-Terpstra Test). Thus, *Atm* nullizygosity has an effect at least partially independent of the genomic instability caused by IR.

To probe further the effects of genomic stability on intestinal tumors, we contrasted *Atm* mutants with a mutant for the *Blm* gene. In agreement with other observations (Luo *et al.*, 2000), homozygosity for a *Blm* hypomorphic allele (Blm^{m3}) increased $Apc^{Min/+}$ tumor multiplicity 3.8-fold (Table 1b). $Blm^{m3/+}$ heterozygotes and homozygous wildtypes did not differ significantly from each other ($p>0.3$). No studies have yet been reported for *Blm* and $Apc^{1638N/+}$; we found that, in contrast to *Atm*-deficiency, *Blm*-deficiency had only a 5-fold enhancing effect on $B6.Apc^{1638N/+}$ tumor multiplicity (Table 1b). Importantly, the 95% CI for the fold-increase caused by *Blm*-deficiency does not overlap with the larger effect caused by *Atm*-deficiency. Furthermore, only one of 20 $B6.Blm^{m3/m3};Apc^{1638N/+}$ mice developed colonic tumors, which is not statistically different from heterozygotes or wildtype controls ($p>0.4$) but is significantly different from $Atm^{-/-};Apc^{1638N/+}$ ($p<0.01$). This *Blm* hypomorph is known to increase $Apc^{Min/+}$ tumor multiplicity via an increase in somatic recombination proximal to the *Apc* locus (Luo *et al.*, 2000; Goss *et al.*, 2002). Therefore, our results with the Blm^{m3} allele argue that the generation of $Apc^{1638N/1638N}$ cells is not sufficient to induce adenomas at a high rate. Thus, although genome-wide somatic hyperrecombination strongly enhances $Apc^{Min/+}$-induced tumors, it is insufficient to explain the dramatic affect of *Atm* loss on $Apc^{1638N/+}$ tumorigenesis.

Finally, we sought further evidence for an effect of Atm deficiency distinct from genomic instability by studying *Apc*-mutant mice haploinsufficient for *Lig4*, which encodes a protein involved in maintaining genomic stability and which, unlike some modifiers of $Apc^{Min/+}$ - Blm (Luo *et al.*, 2000; Goss *et al.*, 2002), Recql4 (Mann *et al.*, 2005), or Bub1R (Rao *et al.*, 2005)- has no known role in cell cycle checkpoints (Frank *et al.*, 2000). $Lig4^{+/-}$ mice have previously been shown to increase the genomic instability and multiplicity of sarcomas in $Ink4a/Arf^{-/-}$ mice (Sharpless *et al.*, 2001). By contrast, $B6.Lig4^{+/-};Apc^{Min/+}$ or $B6.Lig4^{+/-};Apc^{1638N/+}$ mice did not have a significantly different tumor multiplicity ($p>0.6$; data not shown) or average size ($p>0.9$) when compared to $Lig4^{+/+}$ controls. Taken together, these and the preceding results suggest that the effect of *Atm* deficiency on intestinal tumorigenesis involves functions beyond the maintenance of genomic stability.

Atm encodes a protein with pleiotropic functions involving cell cycle checkpoints, DNA double strand break repair, senescence, and apoptosis. It has recently been proposed that Atm and other checkpoint proteins also serve as barriers to the growth of incipient tumors (Gorgoulis et al., 2005; Bartkova et al., 2005). Various human solid tumors show a sharp increase in an activated form of Atm protein at early stages, signals that are then diminished or lost in later-stage carcinomas. Other recent evidence in human tumors correlates a decrease in colon tumor-specific Atm protein levels (Grabsch *et al*l., 2006) and an increase in *Atm* promoter hypermethylation (Bai *et al*., 2004) with progression to carcinoma and decreased survival. Allelic imbalance at the 11q22–23 interval containing *Atm* is also associated with polyploidy

in colorectal carcinomas (Sugai *et al.*, 2001). Inactivation of the Atm and/or related pathways might therefore be required for tumor progression. We have reported here that ablation of the *Atm* gene significantly sensitizes intestinal epithelia to both tumor multiplicity and growth. These findings, along with the recent studies of Atm knockdown in mouse cell line explants and the inverse correlation between senescence and colorectal tumor progression (Bartkova et al., 2006) lends strong support to the hypothesis that the Atm function can negatively regulate tumor emergence in epithelial tissues. Additionally, heterozygosity for the *Atm* mutation appeared to affect intestinal tumor size (but not multiplicity); *Atm* haploinsufficiency has long been controversially associated with human breast cancer (Renwick et al., 2006).

How exactly does Atm control intestinal tumor multiplicity and size via proliferation? The pleiotropy of *Atm* action makes this a difficult question to answer, and is further complicated by the possibility that Atm could act in a non-cell autonomous fashion, similar to studies implicating stromal p53 loss in mouse models of prostate cancer (Hill *et al.*, 2005). Nevertheless, models of possible pathways can be constructed. For example, p53 protein is phosphorylated directly by the Atm kinase (Westphal *et al.*, 1997); perhaps perturbed p53 signaling, including decreased expression levels of p21, potentiates increased cell survival and/ or proliferation. Indeed, we have shown previously that *p53* nullizygosity on the B6 background increases *Apc*$^{Min/+}$ tumor multiplicity from 32 to 45 tumors (Halberg *et al.*, 2000); however, this is significantly less than the increase from Atm loss ($p<0.05$). Thus, other pathways are likely involved: for example, Atm also regulates cell cycle checkpoints, lying directly upstream of many key effectors such as Chk2 (Matsuoka *et al.*, 2000). In principle, defective checkpoint signaling can result in the deregulation of cell division. Finally, the increase in proliferation is consistent with a loss of senescence induction, as recently proposed for the knockdown of Atm (Bartkova *et al.*, 2006). Confirmation of this speculation will require analysis of senescence markers.

In summary, we have demonstrated that *Atm* can negatively regulate tumorigenesis in epithelial tissues by opposing the proliferation of dysplastic cells, acting independently of effects on genomic stability.

Reference List

Amos-Landgraf JM, Kwong LN, Kendziorski CM, Reichelderfer M, Torrealba J, Weichert J, et al. A target-selected *Apc*-mutant rat kindred enhances the modeling of familial human colon cancer. Proc Natl Acad Sci U S A 2007;104:4036–4041. [PubMed: 17360473]

Bai AHC, Tong JHM, To KF, Chan MWY, Man EPS, Lo KW, et al. Promoter hypermethylation of tumor-related genes in the progression of colorectal neoplasia. Int J Cancer 2004;112:846–853. [PubMed: 15386372]

Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, et al. Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 1996;86:159–171. [PubMed: 8689683]

Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006;444:633–637. [PubMed: 17136093]

Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005;434:864–870. [PubMed: 15829956]

Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, et al. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci U S A 1994;91:8969–8973. [PubMed: 8090754]

Frank KM, Sharpless NE, Gao Y, Sekiguchi JM, Ferguson DO, Zhu C, et al. DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. Mol Cell 2000;5:993–1002. [PubMed: 10911993]

Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907–913. [PubMed: 15829965]

Goss KH, Risinger MA, Kordich JJ, Sanz MM, Straughen JE, Slovek LE, et al. Enhanced tumor formation in mice heterozygous for Blm mutation. Science 2002;297:2051–2053. [PubMed: 12242442]

Grabsch H, Dattani M, Barker L, Maughan N, Maude K, Hansen O, et al. Expression of DNA double-strand break repair proteins ATM and BRCA1 predicts survival in colorectal cancer. Clin Cancer Res 2006;12:1494–1500. [PubMed: 16533773]

Haigis KM, Caya JG, Reichelderfer M, Dove WF. Intestinal adenomas can develop with a stable karyotype and stable microsatellites. Proc Natl Acad Sci U S A 2002;99:8927–8931. [PubMed: 12060718]

Haigis KM, Hoff PD, White A, Shoemaker AR, Halberg RB, Dove WF. Tumor regionality in the mouse intestine reflects the mechanism of loss of Apc function. Proc Natl Acad Sci U S A 2004;101:9769–9773. [PubMed: 15210940]

Halberg RB, Katzung DS, Hoff PD, Moser AR, Cole CE, Lubet RA, et al. Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. Proc Natl Acad Sci U S A 2000;97:3461–3466. [PubMed: 10716720]

Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell 2005;123:1001–1011. [PubMed: 16360031]

Janssen KP, Alberici P, Fsihi H, Gaspar C, Breukel C, Franken P, et al. APC and oncogenic KRAS are synergistic in enhancing Wnt signaling in intestinal tumor formation and progression. Gastroenterology 2006;131:1096–1109. [PubMed: 17030180]

Luo G, Santoro IM, McDaniel LD, Nishijima I, Mills M, Youssoufian H, et al. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. Nat Genet 2000;26:424–429. [PubMed: 11101838]

Luongo C, Dove WF. Somatic genetic events linked to the *Apc* locus in intestinal adenomas of the Min mouse. Genes Chromosomes Cancer 1996;17:194–198. [PubMed: 8946200]

Mann MB, Hodges CA, Barnes E, Vogel H, Hassold TJ, Luo G. Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund-Thomson syndrome. Hum Mol Genet 2005;14:813–825. [PubMed: 15703196]

Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proc Natl Acad Sci U S A 2000;97:10389–10394. [PubMed: 10973490]

Rao CV, Yang YM, Swamy MV, Liu T, Fang Y, Mahmood R, et al. Colonic tumorigenesis in $BubR1^{+/-}Apc^{Min/+}$ compound mutant mice is linked to premature separation of sister chromatids and enhanced genomic instability. Proc Natl Acad Sci U S A 2005;102:4365–4370. [PubMed: 15767571]

Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet 2006;38:873–875. [PubMed: 16832357]

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 1995;268:1749–1753. [PubMed: 7792600]

Sharpless NE, Ferguson DO, O'Hagan RC, Castrillon DH, Lee C, Farazi PA, et al. Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. Molecular Cell 2001;8:1187–1196. [PubMed: 11779495]

Sugai T, Habano W, Uesugi N, Jiao YF, Nakamura S, Yoshida T, et al. Frequent allelic imbalance at the ATM locus in DNA multiploid colorectal carcinomas. Oncogene 2001;20:6095–6101. [PubMed: 11593417]

Taketo MM. Mouse models of gastrointestinal tumors. Cancer Science 2006;97:355–361. [PubMed: 16630131]

Westphal CH, Rowan S, Schmaltz C, Elson A, Fisher DE, Leder P. *atm* and *p*53 cooperate in apoptosis and suppression of tumorigenesis, but not in resistance to acute radiation toxicity. Nat Genet 1997;16:397–401. [PubMed: 9241281]
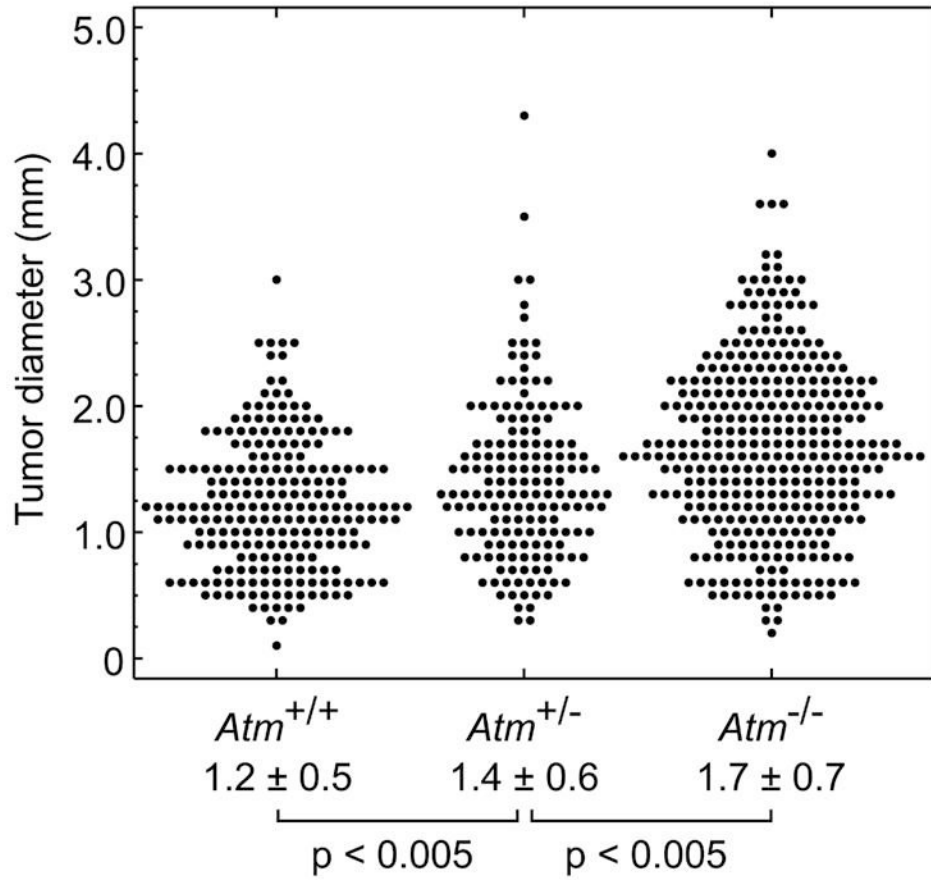
Figure 1.
Effect of Atm genotype on the size of small intestinal tumors in 100–109 day old $Apc^{Min/+}$ mice ($Atm^{+/+}$: n=7 animals, 227 tumors; $Atm^{+/-}$: n=6 animals, 164 tumors; $Atm^{-/-}$: n=5 animals, 353 tumors). Intestinal tumors were measured at the maximal diameter using a dissection microscope with an ocular sizing reticle. Mean ± SD for each set and p-values (Jonckheere-Terpstra test) are shown.
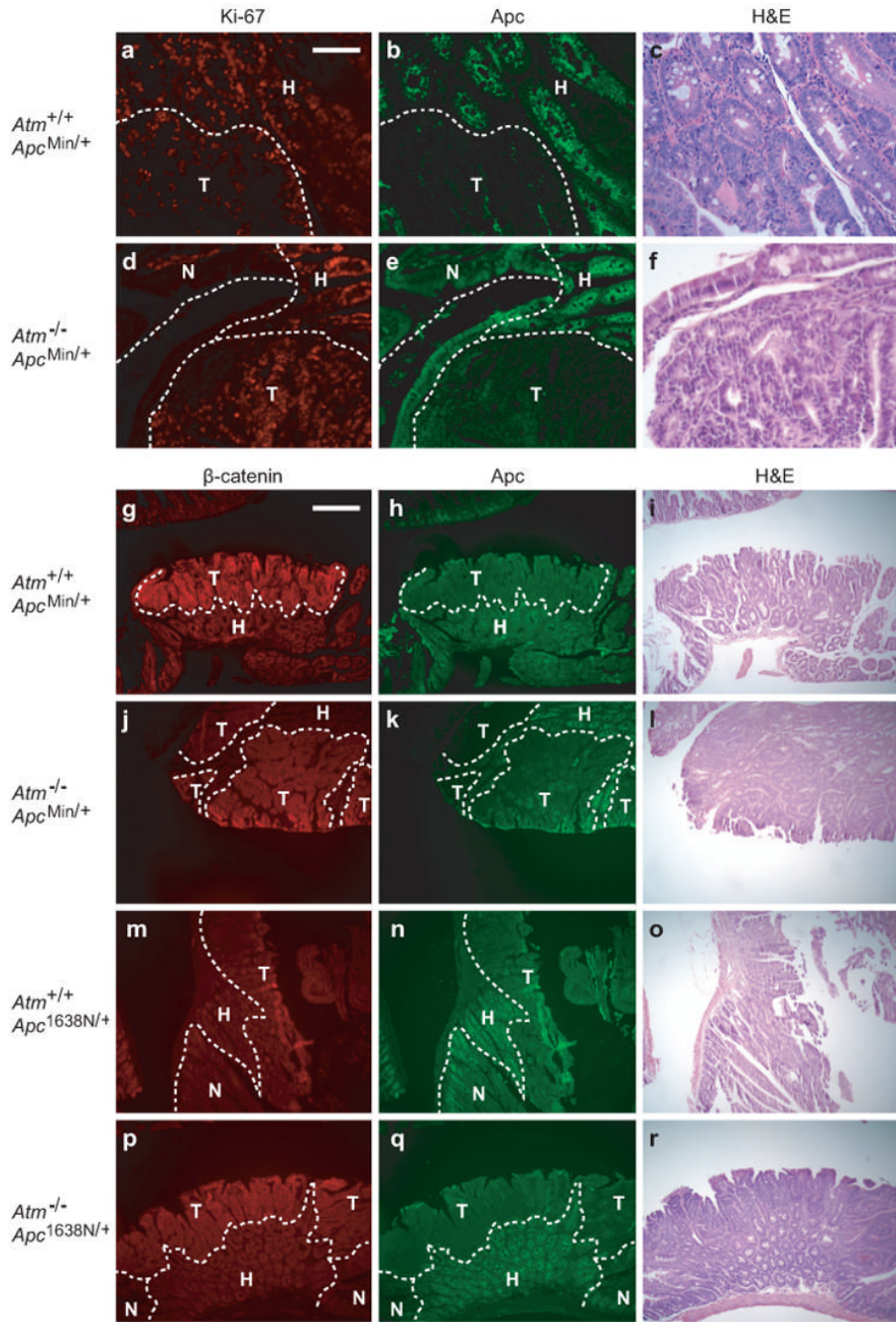
Figure 2.
Ki-67, Apc, and β-catenin staining in intestinal tumors. (**a–c**) $Atm^{+/+};Apc^{Min/+}$ and (**d–f**) $Atm^{-/-};Apc^{Min/+}$ tumors stained for Ki-67 (red), Apc (green) and H&E. (**g–i**) $Atm^{+/+};Apc^{Min/+}$, (**j–l**) $Atm^{-/-};Apc^{Min/+}$, (**m–o**) $Atm^{+/+};Apc^{1638N/+}$, and (**p–r**) $Atm^{-/-};Apc^{1638N/+}$ tumors stained for β-catenin (red), Apc (green) and H&E. Histological classification was determined using Apc levels and cellular morphology. Immunofluorescence was performed as described previously (Haigis *et al.*, 2002). All sections shown are representative. T, tumor; H, tumor-associated hyperplasia; N, normal tissue. Scale bars: a–f, 0.1mm; g–r, 0.4mm.

Table 1

Effect of the genotype of (a) *Atm* and (b) *Blm* on intestinal tumor multiplicity

The approximate fold-increase refers to $Atm^{-/-}$ or $Blm^{m3/m3}$ compared to the other two classes. 129/S6 (129), $Blm^{m3/+}$ mice were a gift from Allan Bradley (Baylor College of Medicine, Houston, TX); 129.$Atm^{+/-}$ mice were purchased from The Jackson Laboratory (Bar Harbor, ME); 129.$Lig4^{+/-}$ mice were a gift from Frederick Alt (Harvard University, Boston, MA). All mice were backcrossed to B6 for at least 11 (Atm), 10 (Blm), or 5 ($Lig4$) generations. Mouse husbandry and dissection were performed as previously described (Haigis *et al.*, 2002). All mice were dissected between 3 and 5 months of age except for irradiated mice, which became moribund at 2 months. Whole-body IR was delivered using a Mark I ^{137}Cs irradiator, model 30 (J L Shepard & Assoc., San Fernando, CA), as previously described (Luongo and Dove, 1996). It was noted that $Atm^{-/-}$;$Apc^{1638N/+}$ and $Blm^{m3/m3}$;$Apc^{1638N/+}$ mice, but no other genotypic combination, developed numerous desmoid fibromas which were not enumerated.

a

	Loc.	$Atm^{+/+}$ Tumor count, mean ± SD (incidence)	N	$Atm^{+/-}$ Tumor count, mean ± SD (incidence)	N	$Atm^{-/-}$ Tumor count, mean ± SD (incidence)	N	Fold Increase (95%CI‡[†])
B6.*Apc*[Min/+]	SI	31 ± 7	47	35 ± 12	49	67 ± 10	17	2.0 (1.8–2.3)*
	LI	0.9 ± 1.5 (47%)		1.1 ± 1.3 (47%)		3.8 ± 3.7 (94%*)		4.6 (2.2–9.3)*
B6.*Apc*[Min/+] +5.0Gy IR	SI	280 ± 70	7	276 ± 54	12	267 ± 53	3	1.0 (0.7–1.3)[†]
	LI	7.7 ± 3.0 (100%)		10 ± 7.5 (100%)		22 ± 5.5 (100%[†])		2.4 (1.4–4.2)*
B6.*Apc*[1638N/+]	SI	1.4 ± 1.1	13	1.5 ± 1.0	21	26 ± 9.4	20	16 (11–22)*
	LI	0 ± 0 (0%)		0 ± 0 (0%)		0.8 ± 0.8 (45%*)		N/A*

b

	Loc.	$Blm^{+/+}$ Tumor count, mean ± SD (incidence)	N	$Blm^{m3/+}$ Tumor count, mean ± SD (incidence)	N	$Blm^{m3/m3}$ Tumor count, mean ± SD (incidence)	N	~Fold-increase (95%CI‡)
B6.*Apc*[Min/+]	SI	28 ± 12	9	25 ± 7	15	99 ± 16	4	3.8 (2.9–5.0)*
	LI	0.1 ± 0.3 (11%)		0.4 ± 0.6 (33%)		1.3 ± 0.5 (100%[†])		4.3 (1.8–21)*
B6.*Apc*[1638N/+]	SI	1.8 ± 0.8	5	0.9 ± 1.1	8	6.2 ± 3.2	20	5.1 (2.8–10)*
	LI	0 ± 0 (0%)		0 ± 0 (0%)		0.1 ± 0.2 (5%[†])		N/A[†]

* p<0.05, Jonkheere-Terpstra Test or χ^2 test compared to controls

[†] No significant difference

[‡] Bootstrap ratios comparing the homozygous knockouts to the combined heterozygotes and wildtypes; N/A, Not Applicable; SI, small intestine; LI, large intestine.

Table 2

The effect of *Atm* deficiency on the proliferative index of *Apc*[Min/+] tumors

The signals were counted, with the observer blind to the genotype, from digital photomicrographs displayed on a computer screen. Only crypts that were clearly epithelial by H&E staining on adjacent serial sections were assessed. Apc staining was used to discriminate between tumor (negative staining) and non-tumor (positive staining) tissue. Cells from the poorly characterized "cap" of Apc-positive cells surrounding the lumenal edges of tumors were not included. Six tumors and adjacent hyperplastic tissue were analyzed per genotype. The p-values were calculated using Fisher's Exact Test.

Tissue type	Ki-67-positive cells/total (percent positive; 95% CI)		P-value
	Atm^{+/+}	*Atm*^{−/−}	
Hyperplastic	481/2163 (22.2%; 20.5–24.1)	424/1461 (29.0%; 27.7–31.4)	<0.01
Tumor	749/2128 (35.2%; 33.2–37.3)	1175/2338 (50.3%; 48.2–52.3)	<0.01