ORIGINAL ARTICLE

Mesalazine and thymoquinone attenuate intestinal tumour development in Msh2loxP/loxP Villin-Cre mice

Benedikt Kortüm,1 Christoph Campregher,1 Michaela Lang,1 Vineeta Khare,1 Matthias Pinter,2 Rayko Evstatiev,1,2 Gerald Schmid,1 Martina Mittlböck,3 Theresa Scharl,4 Melanie H Kucherlapati,5 Winfried Edelmann,6 Christoph Gasche1,2

ABSTRACT

Objective Lynch syndrome is caused by germline mutations in DNA mismatch repair genes leading to microsatellite instability (MSI) and colorectal cancer. Mesalazine, commonly used for the treatment of UC, reduces MSI in vitro. Here, we tested natural compounds for such activity and applied mesalazine and thymoquinone in a Msh2loxP/loxP Villin-Cre mouse model for Lynch syndrome.

Design Flow cytometry was used for quantitation of mutation rates at a CA13 microsatellite in human colon cancer (HCT116) cells that had been stably transfected with pIREShyg2-enhanced green fluorescent protein/CA13, a reporter for frameshift mutations. Mice were treated for 43 weeks with mesalazine, thymoquinone or control chow. Intestines were analysed for tumour incidence, tumour multiplicity and size. MSI testing was performed from microdissected normal intestinal or tumour tissue, compared with mouse tails and quantified by the number of mutations per marker (NMPM).

Results Besides mesalazine, thymoquinone significantly improved replication fidelity at 1.25 and 2.5 μM in HCT116 cells. In Msh2loxP/loxP Villin-Cre mice, tumour incidence was reduced by mesalazine from 94% to 69% (p=0.04) and to 56% (p=0.003) by thymoquinone. The mean number of tumours was reduced from 3.1 to 1.4 by mesalazine (p=0.004) and to 1.1 by thymoquinone (p<0.001). Interestingly, MSI was reduced in normal intestinal tissue from 1.5 to 1.2 NMPM (p=0.006) and to 1.1 NMPM (p=0.01) by mesalazine and thymoquinone, respectively. Thymoquinone, but not mesalazine, reduced MSI in tumours.

Conclusions Mesalazine and thymoquinone reduce tumour incidence and multiplicity in Msh2loxP/loxP Villin-Cre mice by reduction of MSI independent of a functional mismatch repair system. Both substances are candidate compounds for chemoprevention in Lynch syndrome.

INTRODUCTION

Lynch syndrome (LS) also known as hereditary non-polyposis colorectal cancer (CRC) is the most common familial cancer syndrome that predisposes to early onset of CRC and other extracolonic malignancies. It is estimated that LS accounts for 3% of all CRCs.1 The majority of germline mutations in LS families affects genes of the mismatch repair (MMR) system such as Msh2 (60%), Mlh1 (30%) and Msh6 (7%–10%).2 Somatic hyper-methylation of Msh2 and microsatellite instability (MSI) are frequent events in LS CRCs.3 In fact, MSI is found in virtually all Lynch tumours as a result of an accumulation of frameshift mutations at repetitive sequences due to polymerase slippage.4-5 Microsatellites are present throughout the genome mostly in non-coding regions. When present in the coding region of tumour suppressor genes, frameshift mutations in such microsatellites cause protein truncation and loss of function, thereby promoting carcinogenesis.6-7 Mesalazine (5-aminosalicylic acid, 5-ASA), the active moiety of sulfasalazine,8 and structurally related to aspirin, is used for treatment of active UC and maintenance of remission. In general, mesalazine is well tolerated, and most colitis patients take it for several years. Longstanding UC predisposes to...
CRC. Case-control studies have pointed to possible chemopreventive effects of sulfasalazine and mesalazine in the setting of colitis.9,10 The chemopreventive properties of mesalazine were subject of various in vitro studies.10 Mesalazine exerts anti-inflammatory and chemopreventive effects by inhibition of nitric oxide synthase,11 downregulation of cyclooxygenase (COX)-2,12 reactive oxygen species scavenging12 and inhibition of nuclear factor (NF)-κB pathway.13 Furthermore, its involvement in c-myc expression,14 epidermal growth factor receptor,15 peroxisome proliferator-activated receptor (PPAR)-γ16 and Wnt/β-catenin17 signalling by inhibition of p-21 activated kinase 19,18 has been reported. Mesalazine, but none of its derivatives, arrests MMR-deficient human colon cancer (HCT116) cells in the G2-M phase by activation of a replication checkpoint and improves replication fidelity in mononucleotide, dinucleotide and tetranucleotide repeats.19 20 21 22 In this regard, mesalazine seems to be a good candidate for chemoprevention of LS. Also, certain natural compounds are thought to have chemopreventive properties. It is possible that specific natural compounds may also interfere with replication fidelity and may be candidate drugs for chemoprevention of LS.

Here, we tested certain natural compounds for their ability to improve replication fidelity in vitro. The Msh2loxP/loxP Villin-Cre mouse had been established to simulate intestinal carcinogenesis in LS for development of chemopreventive drugs.23 In this transgenic mouse, the Msh2-loxP allele was crossed with the Villin-Cre transgene, achieving an intestinal-specific conditional knockout of Msh2, where the Msh2 allele is excised by Cre-recombinase in Villin-expressing tissue. Msh2loxP/loxP replicative knockout of Msh2, where the Msh2 allele is excised by Cre-recombinase in Villin-expressing tissue. Msh2loxP/loxP signalling by inhibition of p-21 activated kinase 19,18 has been reported. Mesalazine, but none of its derivatives, arrests MMR-deficient human colon cancer (HCT116) cells in the G2-M phase by activation of a replication checkpoint and improves replication fidelity in mononucleotide, dinucleotide and tetranucleotide repeats.20 21 22 23 In this regard, mesalazine seems to be a good candidate for chemoprevention of LS. Also, certain natural compounds are thought to have chemopreventive properties. It is possible that specific natural compounds may also interfere with replication fidelity and may be candidate drugs for chemoprevention of LS.

Here, we tested certain natural compounds for their ability to improve replication fidelity in vitro. The Msh2loxP/loxP Villin-Cre mouse had been established to simulate intestinal carcinogenesis in LS for development of chemopreventive drugs.23 In this transgenic mouse, the Msh2loxP/loxP allele was crossed with the Villin-Cre transgene, achieving an intestinal-specific conditional knockout of Msh2, where the Msh2 allele is excised by Cre-recombinase in Villin-expressing tissue. Msh2loxP/loxP Villin-Cre mouse develop spontaneous intestinal neoplasia within 10 months, predominantly in the small intestine. Here, we also used this Msh2loxP/loxP Villin-Cre mouse to test whether mesalazine or thymoquinone (TQ) interferes with intestinal carcinogenesis in vivo.

MATERIALS AND METHODS

Cells and compounds

HCT116-A2.1, an MMR-deficient MSI-reporter cell line for replication errors within a (CA)13 repeat was grown in Iscove’s Modified Dulbecco’s Medium (Gibco/Invitrogen, Karlsruhe, Germany) containing 200 µg/ml hygromycin B and 10% fetal bovine serum (FBS, Biochrom, Berlin, Germany) at 37°C, 5% CO2 and full humidity.24 Cinnamaldehyde (W228613, Sigma-Aldrich, Germany) for nuclear counterstaining. 1912. doi:10.1136/gutjnl-2014-307663

Histology and immunohistochemistry

The paraffin-embedded Swiss rolls were cut in three layers (upper, middle, lower levels). In each level, five sections of 5 µm thickness were collected, one for H&E staining, two sections mounted on polyethylene naphthalate (PEN) membrane slides and subsequently stained with Methyl Green for laser capture microdissection and two sections mounted on sialinated slides, left unstained and in paraffin-embedded state for subsequent histochemical examinations. These serial tissue sections were analysed for tumour multiplicity, incidence and size by two independent researchers who were blinded for the treatment group (BK and MP). Tumour size was scored as small, medium or large according to its visibility on 1, 2 or all 3 sections of the block (see online supplementary figure S1). Immunohistochemistry was performed using antibodies against Msh2 (IHC-00082, Bethyl Laboratories, Montgomery, Texas, USA; 1:250), Cre-recombinase (BIOT-106L, Covance, Princeton, New Jersey, USA; 1:100) and Ki-67 (ab15580, abcam, Cambridge, UK; 1:800) diluted in 10% normal goat serum (VECTOR S1000, Vector Laboratories, Peterborough, UK) or 2% horse serum (VECTOR S2000) and 3% bovine serum albumin (BSA) following standard protocols.25 After removing paraffin from the heat-dried tissue slides, the sections were rehydrated in a descendent ethanol row and water, followed by blocking of endogenous peroxidase with methanol +15% H2O2. Antigen retrieval was performed by boiling the slides in citrate buffer (pH 6). Before antibody use, the tissue sections were blocked with 10% goat serum or 2% horse serum and 3% BSA. Staining was visualised by applying respective secondary antibodies and avidin-biotin-horseradish peroxidase complex (VECTASTAIN Elite ABC Kit, Vector Laboratories), 3,3 ′-diaminobenzidine (32750; Sigma-Aldrich, St Louis, Missouri, USA) and haematoxylin (5174, Merck, Darmstadt, Germany) for nuclear counterstaining.

For analysis of Ki-67 staining, a colour deconvolution plug-in (ImageJ, NIH, USA, V.1.43s) was run to split channels into a brown (Ki-67) and blue (haematoxylin) image. The brown images were converted into an eight-bit greyscale image, a threshold was set to represent Ki-67 positive cells only and the area fraction of positive cells in percentage was calculated.
Laser capture microdissection and fragment analysis

Tissue sections mounted on PEN membrane slides (Leica Microsystems, Wetzlar, Germany) were dissected using the LMD6000 laser microdissection microscope (Leica) to obtain samples of normal mucosa and tumour tissue. DNA was extracted from the microdissected samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, The Netherlands) according to the instructions of the manufacturer.

To examine MSI in tissue samples, six different microsatellite loci as recommended by Kabbarah et al.28 were amplified by PCR using the Multiplex PCR Kit (Qiagen), and fluorescent-labelle

Continuous data are described by means and SDs in the case of normally distributed data, and by medians, minimum and maximum otherwise. Categorical data are described by frequencies and percentages. Mutation rates in HCT116-A2.1 were calculated as previously described using the method of the mean.29 Tumour incidence due to unrelated reasons (two in the untreated group, one each in the TQ high and the 5-ASA low groups). Overall, weight gain was regular except for the male population, in which treatment with 5-ASA high (p=0.002) or TQ high (p=0.010) strongly reduced cell growth (IC50 94 μM), while taurine did not affect cell growth (IC50 250 μM) and taurine at (10 or 15 mM; table 1). TQ reduced cell growth at both concentrations tested (IC50 1.2 μM) and improved replication fidelity when used at a concentration of 2.5 μM (table 1). Therefore, we decided to use TQ and mesalazine for chemoprevention of intestinal neoplasia in Msh2loxP/loxP Villin-Cre mice.

RESULTS

TQ improves replication fidelity

We previously found that mesalazine and certain mesalazine derivatives improve replication fidelity independent of a functional MMR system.26 In a search for natural compounds, which may have similar properties, we tested cinnamaldehyde, taurine and TQ for their effect on replication fidelity within a CA13 dinucleotide-repeat in MMR-deficient HCT116-A2.1 cells. Twenty-four hours after sorting 2500 EGFP-negative cells into 24-well plates, cinnamaldehyde, taurine and TQ were added at different concentrations for 7 days. Cinnamaldehyde strongly reduced cell growth (IC50 94 μM), while taurine did not affect cell growth (figure 1). No effect on replication fidelity was observed by treatment with cinnamaldehyde (at 125 or 250 μM) and taurine at (10 or 15 mM; table 1). TQ reduced cell growth at both concentrations tested (IC50 1.2 μM) and improved replication fidelity when used at a concentration of 2.5 μM (table 1).

Mesalazine and TQ reduce intestinal tumour incidence and multiplicity

Msh2loxP/loxP Villin-Cre mice were genotyped (figure 2A) and characterised for expression of Cre-recombinase (figure 2B) and loss of Msh2 expression in intestinal epithelium (figure 2C). Cre-recombinase was expressed exclusively in intestinal epithelial cells, in which Msh2 expression was also lost. Mice were treated with two concentrations of mesalazine (5-ASA low and 5-ASA high) or TQ (TQ low and TQ high) for 43 weeks. Totally, four mice died within 20 weeks of treatment due to unrelated reasons (two in the untreated group, one each in the TQ high and the 5-ASA low groups). Overall, weight gain was regular except for the male population, in which treatment with 5-ASA high (p=0.002) or TQ high...
(p=0.007) resulted in less weight gain compared with the untreated group (see online supplementary figure S2). This was likely due to a reduced food intake (about 5%–10% less) in the mesalazine and TQ treatment groups compared with the control group. A small group of control mice (n=7) was euthanased every couple of weeks to evaluate tumour development over time. After 20, 24, 34 and 39 weeks of treatment, the small intestinal tumour incidence was 29%, 29%, 71% and 100%, respectively. Tumour multiplicity increased accordingly (see online supplementary figure S3). Both mesalazine and TQ reduced the incidence and multiplicity of tumours in the small intestine. Tumour incidence was reduced from 94% to 69% (p=0.04; RR=0.73; 95% CI 0.58 to 0.93; 5-ASA low: 63%, 5-ASA high: 75%) by mesalazine and from 94% to 56% (p=0.003; RR=0.60; 95% CI 0.44 to 0.80; TQ low: 58%, TQ high: 55%) by TQ (figure 3A). The difference between the compounds was not significant (p=0.25). A dose relationship was observed only for TQ (figure 3A). Tumour multiplicity was reduced from mean 3.1 tumours per mouse to mean 1.4 by 5-ASA (combined p=0.004, 5-ASA low: 1.4, p=0.008; 5-ASA high: 1.5, p=0.018) and to mean 1.1 tumours per mouse by TQ (combined p<0.001, TQ low: 1.4, p=0.008; TQ high: 0.9, p<0.001) (figure 3B). These changes in small intestinal tumour numbers and multiplicity were independent of apoptotic events (see online supplementary figure S4) or any antiproliferative effects (see online supplementary figure S5) in normal and tumour tissue. Both compounds reduced the number of small-sized and medium-sized tumours, but had no effect on large tumour counts, or on small intestinal carcinomas (see online supplementary figure S6).

Msh2loxP/loxP Villin-Cre mice also displayed some single caecal adenomas and caecal carcinomas, while we observed no other colonic tumour (figure 3C). Both mesalazine and TQ showed a tumour reduction from 39% in untreated mice to 21% (p=0.19; RR: 0.53, 95% CI 0.23 to 1.23) and to 31% (p=0.56; RR: 0.79, 95% CI 0.38 to 1.67), respectively, which did not reach statistical significance. The present results suggest a chemopreventive effect of mesalazine and TQ on small intestinal and caecal tumorigenesis in Msh2loxP/loxP Villin-Cre mice independent of induction of apoptosis.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Method of the mean</th>
<th>Maximum likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (%)</td>
<td>Treated (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control (%)</td>
<td>Treated (%)</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>125 µM</td>
<td>6.1±1.8</td>
<td>6.6±2.4 (108)</td>
</tr>
<tr>
<td></td>
<td>250 µM</td>
<td>8.1±3.9 (132)</td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>10 mM</td>
<td>2.9±0.9</td>
<td>3.5±1.0 (120)</td>
</tr>
<tr>
<td></td>
<td>15 mM</td>
<td>4.5±1.3 (154)</td>
<td></td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>1.25 µM</td>
<td>6.3±1.8</td>
<td>5.2±1.6 (82)</td>
</tr>
<tr>
<td></td>
<td>2.50 µM</td>
<td>3.0±1.0* (47)</td>
<td></td>
</tr>
<tr>
<td>Mesalazine†</td>
<td>5 mM</td>
<td>6.0±1.1</td>
<td>4.9±1.4* (82)</td>
</tr>
</tbody>
</table>

*Note: data (×10⁻⁴) are means±SE of mutation rates per microsatellite per generation. Changes are indicated in (%) treated versus control.

*p<0.05.

†Data from reference.22

**Figure 2** Genotyping of conditional Msh2 knock-out. (A) Genotyping of tail DNA for conditional knock-out of exon 12 of the Msh2 gene. DNA from wildtype mice gave a 210 base pair product, whereas transgenic mice (#1–7) yielded a 405 base pair product. (B) Expression of Cre recombinase in nuclei of intestinal epithelial cells of Msh2loxP/loxP villin-Cre mice. (C) Msh2 expression is depleted in intestinal epithelial cells but not in pancreatic tissue and lamina propria (arrow) from Msh2loxP/loxP Villin-Cre mice (left). (D) Control mice (wildtype) express Msh2 mainly in the crypts.


Downloaded from http://gut.bmj.com/ on July 7, 2017 - Published by group.bmj.com
Mesalazine and TQ reduce MSI in Msh2-deficient epithelium

As mesalazine and TQ reduce the mutation rate at microsatellites in vitro, we intended to measure microsatellite mutations from intestinal epithelial cells and tumour tissue after microdissection. After PCR amplification of a panel of previously established murine microsatellite markers, frameshift mutations were quantified as NMPM in comparison with tail DNA (figure 4A). Frameshift mutations were already observed in normal small intestinal enterocytes as they lack Msh2 (figure 2). The most sensitive marker to the treatment was CT25CA27 (table 2). In normal small intestinal epithelium, a consistent reduction in NMPM was observed in all treatment groups, which was significant for 5-ASA high, TQ-low and TQ-high (figure 4B). In tumour tissue, the frequency of mutations in untreated mice was significantly higher than in normal tissue (tumour: 2.4±0.12 NMPM normal: 1.49±0.09 NMPM; p<0.001, table 2). A significant reduction within tumour tissue was observed only by TQ (2.4±0.12 NMPM to 1.47±0.39 NMPM; p=0.016) (figure 4C, table 2).

**DISCUSSION**

LS is caused by germline mutations in certain MMR genes, which, when the according wildtype allele is lost, result in MMR deficiency and MSI. An MMR-independent improvement of replication fidelity is an ideal mechanism for chemoprevention of LS as it lowers the mutation rate and delays the progression to cancer. Mesalazine has been shown to improve replication fidelity in vitro,12 19 20 Here, we identified similar properties of TQ, an extract from *Nigella sativa*, and further advanced both compounds to in vivo tests in a LS mouse model with intestine-specific loss of Msh2. Both mesalazine and TQ reduced tumour incidence and multiplicity in this Msh2<sup>loxP/loxP</sup> Villin-Cre mouse. As hypothesised, the prevention of intestinal carcinogenesis was accompanied by a reduction of frameshift mutations in intestinal epithelial cells, a result of improved replication fidelity independent of the presence of Msh2. Such antimutation effect of mesalazine was not observed in tumour tissue, indicating that the chemopreventive properties of this compound can act only before tumour initiation. Low-dosed mesalazine was also more effective than at high dosage, a finding that warrants further investigation. One explanation could be elevated intracellular oxidative stress induced by mesalazine’s activity on mitochondrial transmembrane potential.30

Aspirin and non-steroidal anti-inflammatory drugs (NSAID) may prevent colorectal carcinogenesis in familial adenomatous polyposis and sporadic CRC.31 Such types of CRC are driven by adenomatous polyposis coli (APC) mutations and chromosomal instability. In LS, however, CRC is driven by MSI. In a large clinical trial, aspirin did not reduce the risk for intestinal neoplasia at the primary endpoint.32 Such proposed effect was observed only after several years in a posthoc analysis.33 Also, in vitro data on aspirin are conflicting: it does not improve replication fidelity,22 but was reported to reduce the accumulation of microsatellite mutations in MMR-deficient cells by genetic selection.34 Aspirin had no effect on intestinal tumorigenesis in Msh2<sup>loxP/loxP</sup> Villin-Cre mice.35 Mesalazine has structural similarity to aspirin, but has almost opposite biological and clinical effect. Mesalazine improves replication fidelity at mononucleotide, dinucleotide and trinucleotide repeats in vitro leading to reduction of MSI,12 11 it increases enterocyte adherence and reduces intestinal permeability,36 37 it is a rather weak COX-inhibitor, and arrests cells in S-phase rather than in G0/G1.19 36 37 It is used as first-line treatment for UC, while aspirin and NSAIDs rather worsen colitis symptoms. Certain mesalazine effects even depend on the exact position of the amino group.12 20 Lastly, mesalazine has minimal systemic effects and almost no adverse reactions.

**TQ** is the major phytochemical constituent of the volatile seed oil of *N sativa*, also called black cumin, which is widely used in herbal medicine. Studies have demonstrated
anti-inflammatory, antioxidant, anticarcinogenic and antiproliferative effects in various cancers, including CRC, where it induces apoptosis through suppression of NF-κB, activation of Akt, induction of the potent tumour suppressor PPAR-γ, upregulation of p53 and p21WAF and inhibition of tumour angiogenesis. In APCMin mice, TQ induced tumour-specific apoptosis. In the Msh2loxP/loxP Villin-Cre mouse, we were unable to observe such increase in apoptosis both in normal and tumour tissue. One explanation might be that induction of apoptosis upon TQ might depend on Msh2. Interestingly, both compounds did not reduce proliferation of intestinal epithelial cells, but rather, increased the fraction of Ki-67-positive intestinal epithelial cells. This goes in line with mesalazine’s induction of an S-phase check point, which increases the time of replication leaving more cells as Ki-67 positive. The chemopreventive activity of TQ in this mouse model was superior to APCMin mice indicating that induction of apoptosis is not its main mechanism of action, but rather, improvement of replication fidelity as shown in vitro, and by fragment analysis of microdissected tissue.

In this study, a substantial incidence of caecal tumours was found that had not been reported earlier. We cannot rule out that such tumours were under-reported in the first place, but a change in tumour expression may also be caused by different housing conditions or different chow. The effects of both compounds on such caecal tumours were less pronounced than on small intestine and did not reach statistical significance as the total numbers were small. Contrary to the small intestine, mesalazine performed better than TQ, possibly due to different pharmacokinetics. As both compounds were part of the chow, translation of our data into the human setting is difficult, as a delayed-release delivery has not been tested. It is to say, that neither substances showed significant effect on large tumours of the small intestine, which have likely been the first to develop, presumably within the first couple of weeks of life before the mice where submitted to treatment. This finding

Figure 4 Mesalazine (5-ASA) and thymoquinone (TQ) reduce microsatellite instability (MSI) in microdissected tissue. (A) Raw data from a dinucleotide and a poly-A mononucleotide marker visualised with GeneMapper software. Major allele sizes of normal intestinal epithelium and tumour cells were compared with tail DNA. MSI was reduced by 5-ASA and TQ in Msh2-deficient epithelium. MSI quantification by number of mutations per marker (NMPM) in Msh2-deficient epithelium (B) and tumour cells (C). Significance was illustrated as *p<0.05, **p<0.01.
suggests that both substances are more efficient in preventing cancer initiation than delaying its progression. Here, we demonstrate that improvement of replication fidelity can be achieved in vitro and in vivo, which results in deceleration of MSI. Importantly, the previously established mathematical model for calculation of mutation rates in vitro is generation-based and, therefore, a reduction of cell proliferation can be ruled out for being responsible for the observed improvement of replication fidelity. Furthermore, similar cell lines revealed decreased replication fidelity upon polymorphonuclear neutrophils-induced stress paralleled by reduced cell proliferation.

Others have shown that mesalazine and TQ induce apoptosis dependent on dose, duration and type of cell line. At the concentrations in our in vitro experiments, cell viability is maintained and induction of apoptosis has not been observed. Furthermore, Msh2 is necessary for the induction of a G2/M checkpoint arrest after replication errors and for induction of DNA repair or apoptosis. The deletion of Msh2 in intestinal epithelial cells could be a reason for the resistance against apoptosis-inducing effects upon treatment with mesalazine and TQ.

The clinical management of LS mutation carriers has been the subject of controversies, one of which is the regular use of fluoroouracil treatment of LS tumours has been reconsidered.

<table>
<thead>
<tr>
<th>Microsatellite marker</th>
<th>Chromosome</th>
<th>Gene Bank</th>
<th>Tissue</th>
<th>Untreated</th>
<th>5-ASA low</th>
<th>5-ASA high</th>
<th>TQ low</th>
<th>TQ high</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG27</td>
<td>1085.1</td>
<td>SI epithelium</td>
<td>1.13 (0.09)</td>
<td>0.88 (0.08)</td>
<td>1.06 (0.06)</td>
<td>0.94 (0.10)</td>
<td>0.94* (0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC098712</td>
<td>SI tumour</td>
<td>2.60 (0.31)</td>
<td>2.00 (0)</td>
<td>3.33 (0.99)</td>
<td>1.22 (0.22)</td>
<td>0.83 (0.17)</td>
<td></td>
</tr>
<tr>
<td>GA9</td>
<td>5G1</td>
<td>SI epithelium</td>
<td>1.56 (0.20)</td>
<td>1.06 (0.10)</td>
<td>1.33 (0.14)</td>
<td>1.24 (0.16)</td>
<td>1.18 (0.15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC083948</td>
<td>SI tumour</td>
<td>3.40 (0.31)</td>
<td>3.20 (1.36)</td>
<td>5.00 (1.13)</td>
<td>1.56 (0.41)</td>
<td>1.67 (0.42)</td>
<td></td>
</tr>
<tr>
<td>CT25G207</td>
<td>3A2</td>
<td>SI tumour</td>
<td>1.19 (0.10)</td>
<td>0.88 (0.15)</td>
<td>0.61 (0.12)</td>
<td>0.81 (0.14)</td>
<td>0.94 (0.13)</td>
<td></td>
</tr>
<tr>
<td>GA14</td>
<td>AC079442</td>
<td>SI tumour</td>
<td>2.40 (0.4)</td>
<td>2.40 (0.98)</td>
<td>3.33* (0.67)</td>
<td>1.88 (0.70)</td>
<td>1.17 (0.31)</td>
<td></td>
</tr>
<tr>
<td>A93</td>
<td>17E4</td>
<td>SI epithelium</td>
<td>1.38 (0.15)</td>
<td>1.18 (0.15)</td>
<td>0.89 (0.20)</td>
<td>0.94 (0.23)</td>
<td>1.06 (0.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC096777</td>
<td>SI tumour</td>
<td>1.10 (0.10)</td>
<td>1.60 (0.60)</td>
<td>1.67 (0.33)</td>
<td>1.00 (0.33)</td>
<td>1.17 (0.65)</td>
<td></td>
</tr>
<tr>
<td>A17</td>
<td>17E4</td>
<td>SI epithelium</td>
<td>2.19 (0.21)</td>
<td>1.94 (0.16)</td>
<td>1.89 (0.18)</td>
<td>1.81 (0.26)</td>
<td>1.65 (0.21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC096777</td>
<td>SI tumour</td>
<td>2.50 (0.34)</td>
<td>2.40 (0.40)</td>
<td>3.83 (0.40)</td>
<td>2.78 (0.52)</td>
<td>2.50 (0.67)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean (SEM).
*p<0.05,  †p<0.01.
5-ASA, 5-aminosalicylic acid; NMPM, number of mutations per marker; SI, small intestinal; TQ, thymoquinone.

Acknowledgements This study was supported by the Austrian Science Fund (P24121 to CG) and the Christian Doppler Research Association. The financial support by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development is gratefully acknowledged. Dr Christoph Kornauth instructed BK into the technique of laser capture microdissection, which was carried out at the Clinical Institute for Pathology, Medical University Vienna. Claudia Wilheim and Elisabeth Eder provided valuable technical expertise and help. Fluorescence images were taken in cooperation with the Imaging Core Facility of the Medical University of Vienna.

Contributors (1) Conception and design of the study: CG, CC, GS. (2) Generation, collection, assembly, analysis and/or interpretation of data: BK, CC, ML, RE, MP, MM, TS, MK, WE. (3) Critical revision of the manuscript: BK, CC, GS, MM, TM, HS, MK, WE, VK, GS, CG.

Funding The financial support by the Federal Ministry of Economy, Family and Youth, and the National Foundation for Research, Technology and Development is gratefully acknowledged. This study was supported, in part, by the Austrian Science Fund (FWP24121 to CG).

Competing interests CG had research collaboration with Giuliani, Shire Pharmaceuticals, Biogena GmbH, AOP Pharmaceuticals and received research support, lecturing or consulting honoraria from Tillotts, Ferring and Dalk Pharma.

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

REFERENCES


12 Campregher C, Luciani MG, Biesenbach P, et al. The position of the aminogroup on the benzene ring is critical for mesalazine’s improvement of replication fidelity. *Inflamm Bowel Dis* 2010;16:576–82.


Mesalazine and thymoquinone attenuate intestinal tumour development in Msh2 Villin-Cre mice

Benedikt Kortüm, Christoph Campregher, Michaela Lang, Vineeta Khare, Matthias Pinter, Rayko Evstatiev, Gerald Schmid, Martina Mittlböck, Theresa Scharl, Melanie H Kuchlerlapati, Winfried Edelmann and Christoph Gasche

Gut 2015 64: 1905-1912 originally published online November 26, 2014
doi: 10.1136/gutjnl-2014-307663

Updated information and services can be found at:
http://gut.bmj.com/content/64/12/1905

These include:

Supplementary Material
Supplementary material can be found at:
http://gut.bmj.com/content/suppl/2014/11/26/gutjnl-2014-307663.DC1

References
This article cites 43 articles, 14 of which you can access for free at:
http://gut.bmj.com/content/64/12/1905#BIBL

Open Access
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Editor’s choice (120)
Open access (355)
Colon cancer (1547)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/